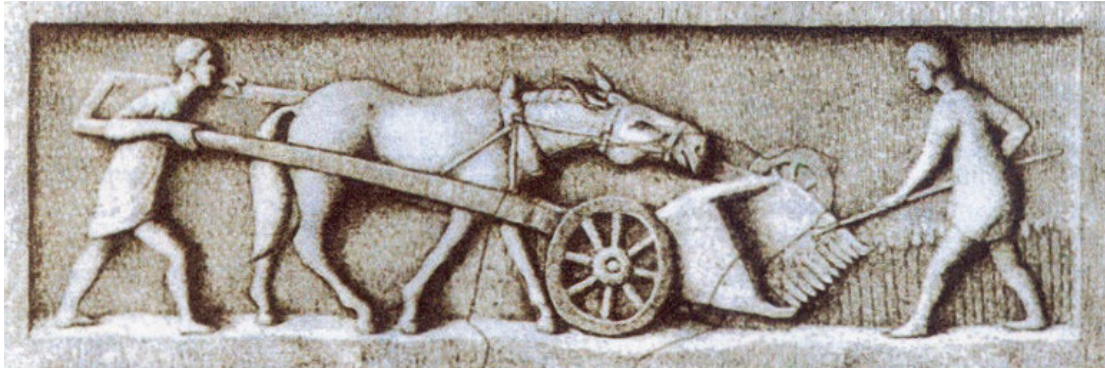


Volume 2, Number 1 (2015)

# Columella

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Dr. POSTA Katalin

Edited by:  
KENDE Zoltán

Responsible publisher:  
LAJOS Mihály

Editorial correspondence:  
Faculty of Agricultural and Environmental Sciences of the Szent István University

Páter Károly utca 1.  
H-2100 Gödöllő, Hungary  
Phone: (+36 28) 522067  
Fax: (+36 28) 410-804  
web: [www.columella.mkk.szie.hu](http://www.columella.mkk.szie.hu)  
E-mail: [columella@mkk.szie.hu](mailto:columella@mkk.szie.hu)

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**Editor-in-Chief**  
Katalin POSTA

**Guest editor**  
Márton JOLÁNKAI

**Edited by**  
Zoltán KENDE

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HUNGARY

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Péter CSATHÓ	Nikolett MAZSU
Goran DJURASINOVIC	Eszter MURÁNYI
Renáta DULOVICHOVÁ	Valéria NAGY
Petr DUŠEK	Mírta RASTIJA
Dario ILJKIC	Rezica SUDAR
Mariann KAMUTI	Gergely SZILÁGYI
Erzsébet KISS	Zsófia TÓTH
Ilija KOMLJENOVIC	Ivana VARGA
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## Table of Contents

Foreword	
Explore, experiment, explain .....	5
Soil, as a multifunctional natural resource	
György VÁRALLYAY.....	9
Response of maize and wheat to fertdolomite application	
Vlado KOVACEVIC – Mirta RASTIJA – Rezica SUDAR – Dario ILJKIC – Ivana VARGA.....	21
Response of maize to liming and phosphorus fertilization with emphasis on weather properties effects	
Ilija KOMLJENOVIC – Mihajlo MARKOVIC – Goran DJURASINOVIC – Vlado KOVACEVIC.....	29
Plant genes as sources to improve pathogen resistance of cultivated plants	
Zsófia TÓTH – Erzsébet KISS.....	37
Effect of plant density and row spacing on maize ( <i>Zea mays</i> L.) grain yield in different crop year	
Eszter MURÁNYI.....	57
Utilization of waste frying oils for transport fuel extender – beneficial to the environment	
Valéria NAGY.....	65
Water regime simulation along Gabčíkovo – Topoľníky channel (Vrakúň junction) case study	
Petr DUŠEK – Yveta VELÍSKOVÁ – Renáta DULOVIČOVÁ.....	73
Performance of wheat quality characteristics impacted by precrop and plant nutrition	
Gergely SZILÁGYI.....	79



## FOREWORD

### EXPLORE, EXPERIMENT, EXPLAIN

The definition of research is rather sophisticated. Research is systematic investigation into and study of materials and sources etc. in order to establish facts and reach to new conclusions. More simply – we seek, we learn.



Really – like in the fairy tales – we are traveling on a road leading to our desired destination, the Kingdom of Far, Far away. Are we there yet? No. But step by step we may proceed, we may get closer. Even if we miss the way, we can learn from our failures.

One of the most peculiar mistakes emerged from a most precise research in the 17<sup>th</sup> century. Jan Baptista van Helmont, a Dutch scientist set up an experiment to explore the principles of plant nutrition. Helmont's willow experiment is often presented in its entirety because the description is so brief. Here is the first English translation from 1662 to refer to for the subsequent discussion.

“But I have learned by this handicraft-operation that all Vegetables do immediately, and materially proceed out of the Element of water onely. For I took an Earthen vessel, in which I put 200 pounds of Earth that hadbeen dried in a Furnace, which I moystened with Rainwater, and I implanted therein the Trunk or Stem of a Willow Tree, weighing five pounds; and at length, five years being finished, the Tree sprung from thence, did weigh 169 pounds, and about three ounces: But I moystened the Earthen Vessel with Rain-water, or distilled water (alwayes when there was need) and it was large, and implanted into the Earth, and leatst the dust that flew about should be co-mingled with the Earth, I covered the lip or mouth of the Vessel with an Iron-Plate covered with Tin, and easily passable with many holes. I computed not the weight of the leaves that fell off in the four Autumnes. At length, I again dried the Earth of the Vessell, and there were found the same two hundred pounds, wanting about two ounces. Therefore 164 pounds of Wood, Barks, and Roots, arose out of water onely.”

It was the first scientific plant analysis approach detecting physiology of plant growth. However the experiment had no plausible results, it can be assumed as the dawn of a new period. Hundreds of experienced scientists and learned landlords tried to find the secret of plant growth and development.

Justus von Liebig tried to find a chemical explanation with his classical barrel stave model. Sir John Bennet Lawes had an opposite approach focusing on plant growth. The scientific duel of these gentlemen is well known in the history of science. Both of them were right and

wrong at the same time just because of the diverse methodology applied. Until the invention of Jean Baptiste Boussingault related to the nitrogen cycle no one has ever realised that the only difference of the two conclusions was based on the assessment, whether the chemical processes were considered static or dynamic.

The advice of Hans Molisch the one time professor in plant physiology is pointing to the importance of interdisciplinary cooperation. He wrote as a motto of his handbook the following slogan: “to understand plants, the gardener has to attend the school of a chemist, and the chemist has to get acquainted with the work of a gardener”.

And it is the point where we come to a most important field of scientific research: the explanation. What sort of conclusions can be taken from scientifically accurate research results?

The mission scientific periodicals, like Columella is to provide floor for dissemination and discussion.

As the new editor-in-chief of the periodical I do hope, that the readers of this volume will benefit from the papers presented. Also, we may have a hope that our readers may contribute to the scientific results presented by the authors.

*Katalin Posta*  
editor-in-chief







## SOIL, AS A MULTIFUNCTIONAL NATURAL RESOURCE

György VÁRALLYAY

Institute for Soil Science and Agricultural Chemistry, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, H-1022 Herman Ottó út 15. Hungary. E-mail: g.varallyay@rissac.hu

**Abstract:** Soils represent a considerable part of the conditionally renewable natural resources. Consequently, rational land use and proper soil management are important elements of sustainability and have special significance in the national economy, environment protection, and even in rural development. The main soil functions are as follows:

- reactor, transformer and integrator of the combined influences of other natural resources;
- medium for biomass production, primary food-source of the biosphere;
- storage of heat, water, plant nutrients and wastes;
- high capacity buffer medium, which may prevent or moderate the unfavourable consequences of various stresses;
- natural filter and detoxication system;
- significant gene reservoir, an important element of biodiversity;
- conservator of natural and human heritages;
- basis for constructions.

Society has utilized these functions in different ways (rate, method, efficiency) throughout history, depending on the given natural conditions and socio-economic circumstances. In many cases irrational (misguided) management leads to “soil loss”, functional disturbances and environment deterioration. The prevention or reduction of these unfavourable consequences requires permanent care and efficient control of soil processes.

**Keywords:** soil functions, soil fertility, resilience, soil degradation, control of soil processes

2015 is the “International Year of Soils”. This paper was compiled for and is dedicated to this event.

### Introduction

Each society wishes and tries to create favourable living conditions for its members. “Life quality criteria” are formulated in different ways by various societies or individuals, depending on the given geographical and socio-economic conditions, living standards; national, ethnical and religious traditions; history, policy; age, sex, educational level, position in the social hierarchy; etc. However, there is full agreement in three elements:

- sufficient quantity of healthy, high quality food: food security;
- clean water;
- pleasant environment.

All three are closely related to the **rational use and proper management of natural**

**resources.** The relationships and interactions between resources and the requirements of society are schematically illustrated by Figure 1. (Lal, 2002; Várallyay, 2010a).

### Sustainability and soil resources

Sustainability is a general concept: the management (use and conservation) of the natural resource base, and the orientation of technological and institutional change in such a manner as to ensure the attainment and *continued satisfaction of human needs for present and future generations.* In agriculture the use of efficient, environment-friendly, non-degrading, energy- and material-saving technologies, which are technically appropriate, economically viable and socially acceptable (Greenland and Szabolcs, 1993, Láng et al., 1983, Várallyay, 2003).

Figure 1. Relationships between resources and the society

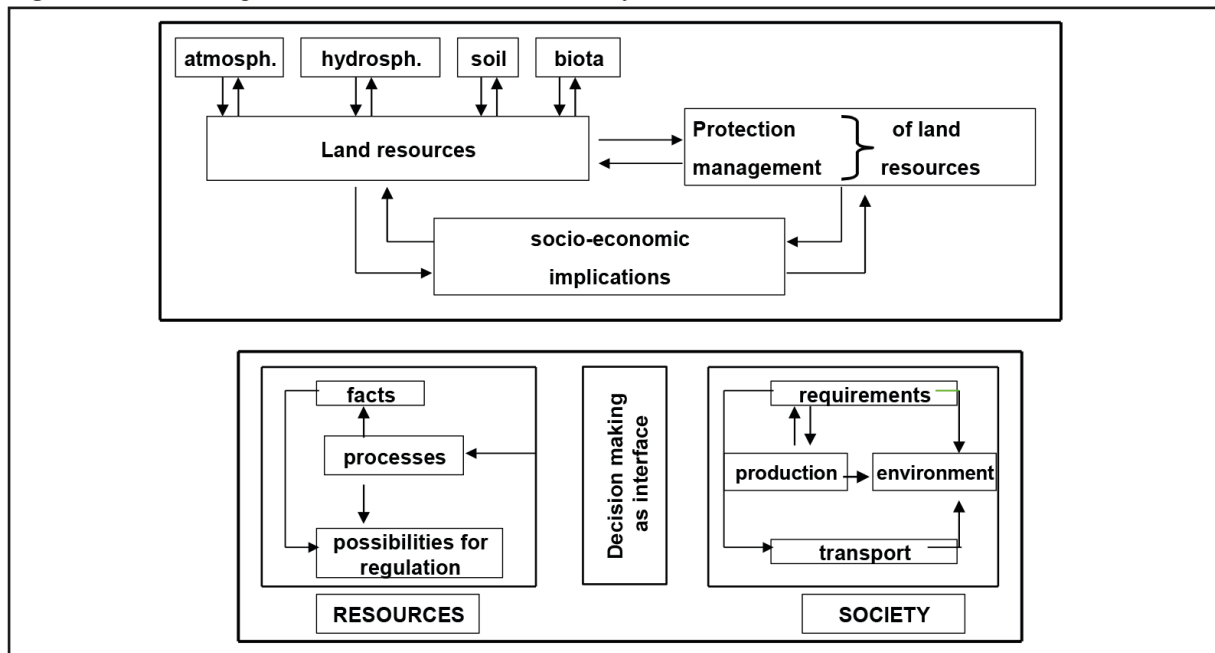
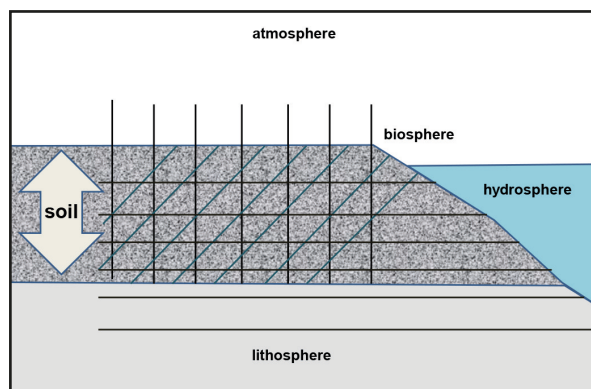


Figure 2. Soil formation by spheric interactions



Soil formed in the interaction zone of the lithosphere, atmosphere, hydrosphere and biosphere (Figure 2) (Németh et al., 2005, Várallyay, 2010a). Soil has three specific/unique characteristics (Greenland and Szabolcs, 1993, Németh et al., 2005, Lal, 2002, Várallyay, 1997, 2003, 2010a):

**(1) Conditionally renewable natural resource.**

As a consequence of their rational use they do not change *irreversibly*, they do not disappear, their quantity and quality doesn't decrease unavoidably, totally and fundamentally. But their potential renewal – based on their *resilience* – doesn't occur automatically and requires permanent

quality maintenance and conservation activities: rational land use and cropping pattern, proper agrotechnics, and – in some cases – remediation, reclamation or amelioration.

**(2) Resilience.**

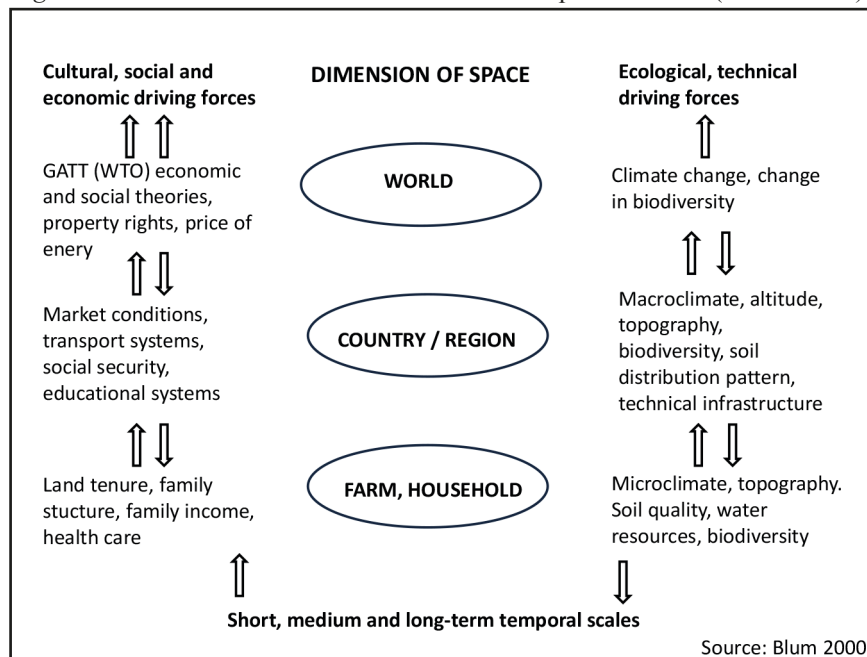
Resilience is „quality or property of (quickly) recovering the original shape and conditions after being stressed (pulled, pressed, crushed, etc.)”.

**Soil resilience** is a specific ability that soil may recover (renew) from various disturbances, natural or human-induced **stresses**. Soil resilience is not a general property of soil. It depends on the soil properties and soil processes, as well as on the character and intensity of the „disturbance”, **stress**, which determine the rate of recovery after „stress-stop” and formulate the tasks to help this **renewal**.

**(3) Multifunctionality.**

Because of these unique characteristics soil is a key factor of sustainability, the possibility and quality of life and social development. The driving forces, spatial and time dimensions of these characteristics are summarized in Figure 3 (Soil Atlas of Europe, 2005).

Figure 3. Land and soil assessment dimensions in space and time (Bloom 2000)



### Soil functions

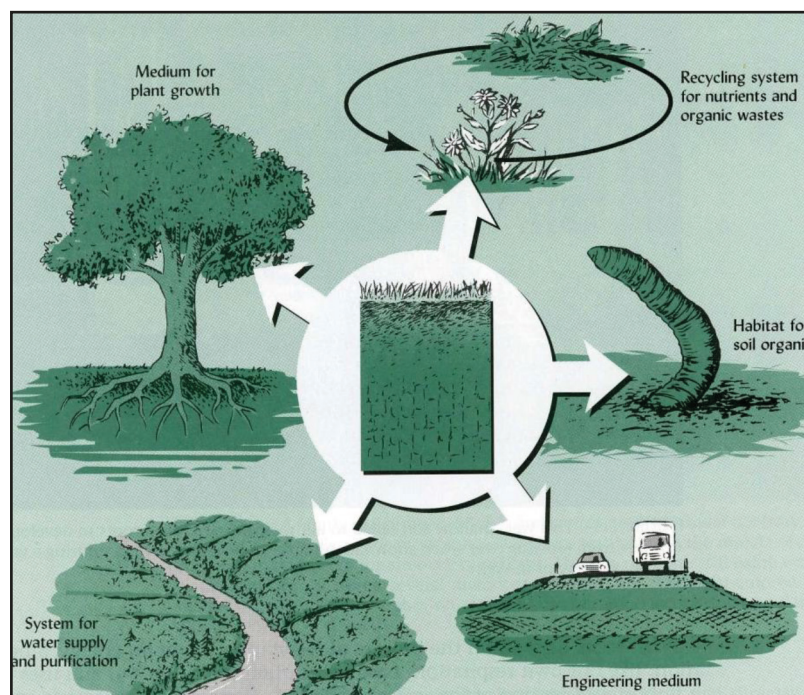
In our present World soil is much more than the most important medium for primary biomass production. Consequently, it has to be managed as a multifunctional natural resource and its other functions have to be taken into consideration in the activities for rational land use and sustainable soil management. The main soil functions in the Global Earth system

are as follows (Figure 4) (Lal, 2002).

1

Soil is a **reactor and transformer**. It integrates the combined influences of other natural resources, such as solar radiation, atmosphere, surface and subsurface waters, deeper geological strata and biological resources. Their biogeochemical cycles develop a “life

Figure 4. Multifunctionality of soil



medium” for microbiological activities, and create ecological environment (standort, landsite) for natural vegetation and cultivated crops (Várallyay, 2000a).

2

Soil is the **most important medium for biomass production** (food, fodder, industrial raw material, alternative energy). Soil, as a four-dimensional [*spatial* (horizontal and vertical) variability and *temporal* dynamism], three- (or four-) phase, polydisperse system and

can simultaneously satisfy – to a certain special extent – the ecological requirements (air, water and nutrient supply) of living organisms, the natural vegetation and cultivated crops (Figure 5). This special ability is the unique soil property: **soil fertility**. It varies greatly and has changed considerably depending on natural factors and human activities (Lal, 2002, Várallyay, 2002b, 2010a).

Soil is the primary food source of the biosphere, the starting point of the food chain (Soil Atlas of Europe, 2005). In addition to these „production” functions, soil has many environmental functions which are summarized in Figure 6 (Lal, 2002, Várallyay, 2000a, 2006).

3

Soils represent a major **natural storage capacity** of heat, water, plant nutrients and – in some special, well-controlled cases – wastes and other materials. The stored water and plant nutrients ensure the *continuous* water and nutrient supply of plants for shorter or longer periods without any additional supply (rain, irrigation, nutrient application). This soil function is the basis of favourable soil moisture

Figure 5. Soil as the medium of biomass

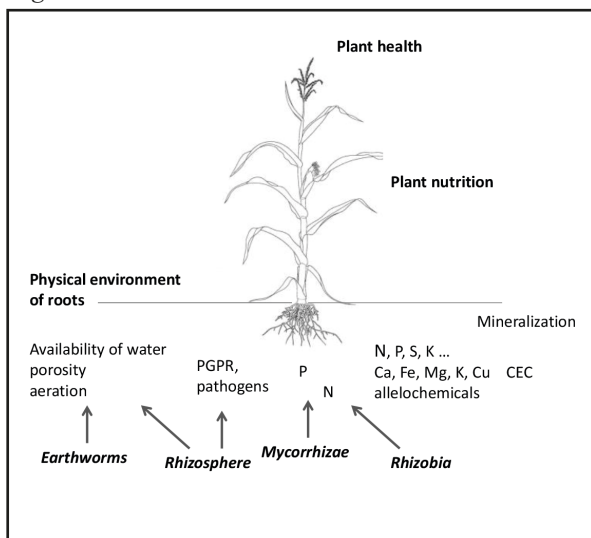


Figure 6. Environmental functions of soil

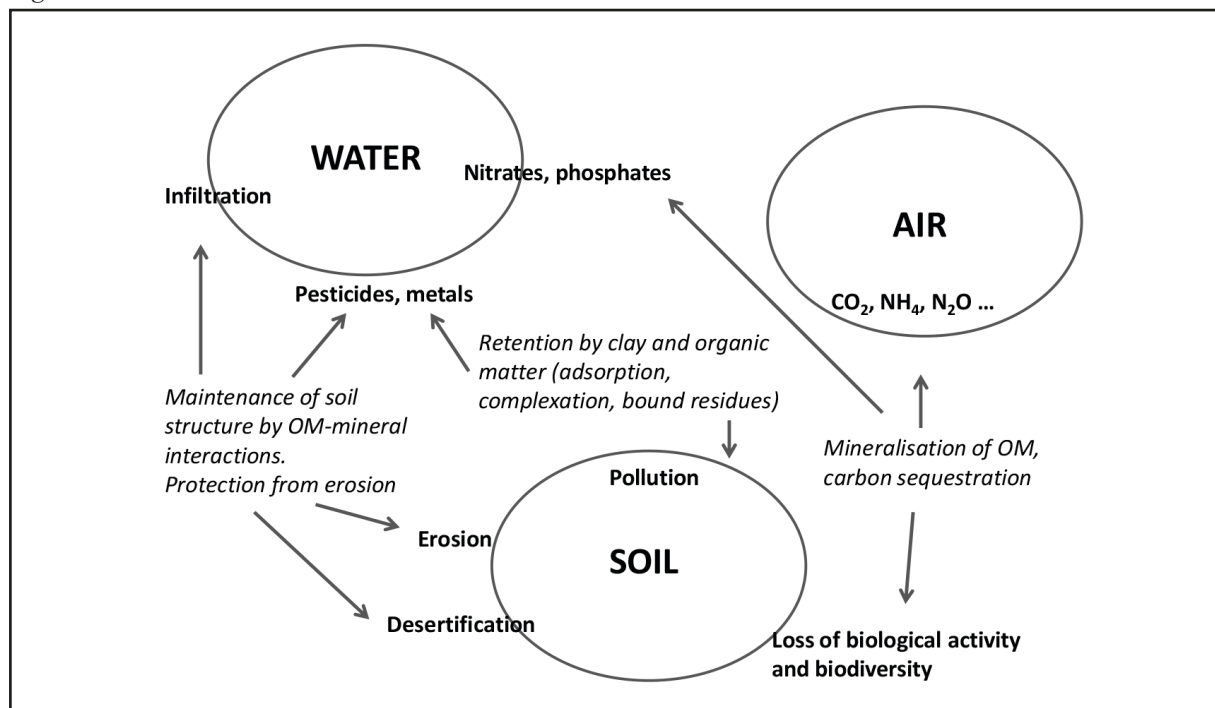


Table 1. Major nutrient elements in soil

	Element	Ionic form taken up	Most important sources	Usual content
<b>Major elements</b>	Nitrogen N	$\text{NO}_3^-$ $\text{NH}_4^+$	organic matter, $\text{N}_2$ from atmosphere	0.03–0.3%
	Phosphorus P	$\text{H}_2\text{PO}_4^-$ $\text{HPO}_4^{--}$ ( $\text{PO}_4^{--}$ )	Ca-, Al-, Fe-phosphate	0.01–0.1%
	Sulphur S	$\text{SO}_4^{--}$	Fe-sulphide, Fe-sulphate	0.01–0.1%
	Potassium K	$\text{K}^+$	micas, illite, K feldspar	0.2–3.0%
	Calcium Ca	$\text{Ca}^{++}$	Ca feldspar, augite, hornblende, $\text{CaCO}_3$ , $\text{CaSO}_4$	0.2–1.5%*
	Magnesium Mg	$\text{Mg}^{++}$	augite, hornblende, olivine, biotite, $\text{MgCO}_3$	0.1–1.0%**

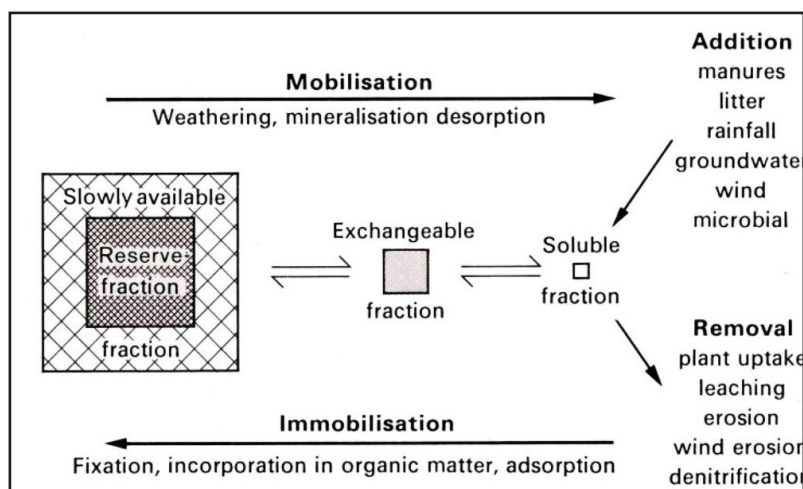
\* Except chalk soils    \*\* Except dolomitic soils

Table 2. Trace elements in soil

	Element	Ionic form taken up	Most important sources	Usual content
<b>Trace elements</b>	Boron B	$\text{H}_2\text{BO}_3^-$ ( $\text{HBO}_3^{--}$ ) ( $\text{B}[\text{OH}]_4^-$ )	tourmaline, accessory in silicates and salts	5–100 ppm
	Molybdenum Mo	$\text{MoO}_4^{--}$	accessory in silicates, Fe- and Al-oxides and hydroxides	0.5–5 ppm
	Chloride Cl	$\text{Cl}^-$	various chlorides	50–> 1000 ppm
	Iron Fe	$\text{Fe}^{++}$ $\text{Fe}^{+++}$	augite, hornblende, biotite, olivine, Fe-oxide and hydroxide	0.5–4.0%*
	Manganese Mn	$\text{Mn}^{++}$ ( $\text{Mn}^{+++}$ )	manganite, pyrolusite, accessory in silicates	200–4000 ppm
	Zinc Zn	$\text{Zn}^{++}$	Zn phosphate, $\text{ZnCO}_3$ , Zn hydroxide, accessory in silicates	10–300 ppm
	Copper Cu	$\text{Cu}^{++}$ ( $\text{Cu}^+$ )	Cu sulphide, Cu sulphate, carbonate, accessory in silicates	5–100 ppm

\* Except Fe enriched horizons

Figure 7. Levels of nutrient availability and mechanisms influencing them



regime and sustainable plant nutrition (Németh et al., 2005, , Várallyay, 2010b). The **water storage function** (Várallyay, 2006, 2010b) has

special significance in the Carpathian Basin, which is a greatly water dependent region. Here the generally favourable natural conditions

show high and irregular (consequently hardly predictable) spatial and temporal variability, often extremes and sensitively react to various natural or human-induced stresses. Due to the irregularity of atmospheric precipitation, the increasing frequency of intense heavy rains („rain bomb”); heterogeneous macro-, meso- and microrelief; unfavourable soil properties; improper land use and cropping pattern the risk, frequency, duration and intensity of **extreme meteorological and hydrological situations** (floods, waterlogging, over-moistening ↔ drought) increase, often in the same year on the same territory.

Under such conditions it is an inevitably important fact that **soil is the largest potential natural water reservoir**. In ideal cases (potentially) in the pore volume of the 0–100 cm soil layer more than half of the 500–600 mm average annual atmospheric precipitation can be stored. About 50% of this quantity is „available moisture content” that may satisfy the water requirement of the natural vegetation and cultivated crops – even at high biomass production and yield levels. In many cases, however, this huge water storage capacity is not used efficiently because of the following limitations:

- limited infiltration (IR);
- limited water retention (FC);

- limited available moisture range (AMR).

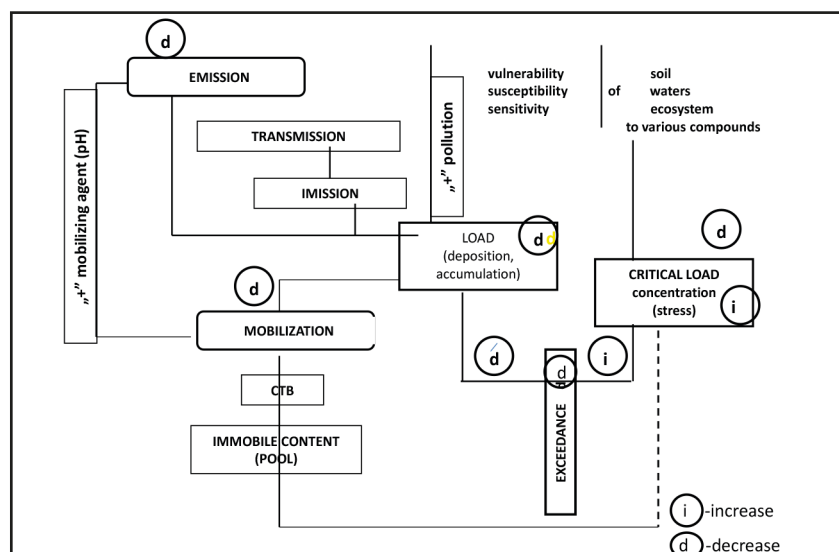
As a consequence of these limitations considerable **water losses** occur by surface runoff, evaporation, seepage and deep filtration. Soils with good agronomic structure may efficiently use the huge water storage capacity of soil and may reduce their unfavourable economical/environmental/social consequences. On the contrary, the infiltration/storage limitations may even *magnify* these threats.

For the illustration of soil’s element storage Table 1 indicates the major nutrient elements and Table 2 the trace elements in soil, showing their usual content and most important sources (Láng et al., 1983, Várallyay, 2010a). Figure 7 schematically summarizes their various forms and their mobilization/ immobilization mechanisms.

4

Soil represents a **high capacity buffer medium** of the biosphere, which, within certain limits, may moderate the various stresses caused by environmental factors (extreme temperature; extreme hydrological events: floods, waterlogging – droughts) and/or human activities (high input, fully-mechanized and chemically controlled crop

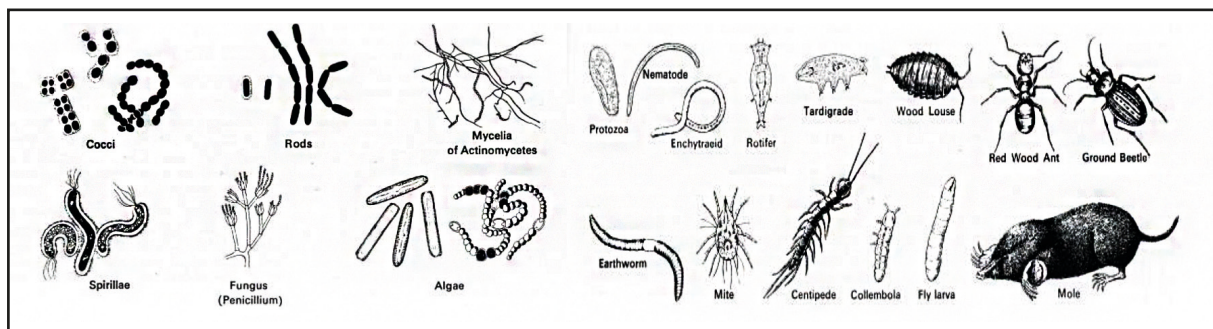
Figure 8. Strategy for soil pollution control





production; liquid manure from large-scale livestock farms; wastes and waste waters originating from industry, transport, urban and rural development, etc.). Buffer systems have strict limits and boundary conditions. Sometimes this is forgotten by the “users”, which leads to serious environmental problems. To prevent and avoid unfavourable side-effects, the *tolerance limits* must be identified, precisely determined, quantified and evaluated. This requires comprehensive sensitivity (susceptibility, vulnerability) studies and impact analyses. Intensive international, regional and national studies have been carried out to determine these tolerance limits and target conditions (Soil Resolution of US, the Integrated European Soil Conservation Strategy and national soil

Figure 9. Living organisms in soil



conservation strategies (Soil Atlas of Europe, 2005) In Hungary comprehensive studies have identified and quantified the susceptibility/sensitivity/vulnerability of soils to wind and water erosion, acidification, salinization/alkalization/ sodification, physical soil degradation (compaction, structure destruction, surface sealing) and chemical pollution (Láng et al., 1983, Lal, 2002, Németh et al., 2005, Várallyay, 2006, Várallyay et al., 2010).

5

Soil is an **efficient „natural filter” and detoxication system** that may prevent the deeper horizons and the subsurface waters from becoming contaminated by various pollutants deposited on the soil surface or put into the

soil. Efficient *soil pollution control* (Figure 8) requires exact and quantitative information on the filter and detoxication „capacity” of soil, on the source and character of the pollutant(s) and on their interaction mechanisms.

6

Soil is a significant **gene reservoir** for the biosphere and thus an **important element of biodiversity**. A considerable proportion of living organisms live in or on the soil or are closely related to (sometimes depend on) the soil. Some examples are shown of these very different (size, shape, life, habitat, role in ecosystems and soil processes) organisms in Figure 9 (Lal, 2002, Soil Atlas of Europe, 2005).

7

Soil is the **conservator of natural and**

**human heritages.**

8

Basis for constructions and other **soil sealing** activities: buildings and „urbanization” infrastructure; roads, highways, railways, aerodroms; water works (reservoirs, ponds, canals, waterways, dykes, other hydrological constructions); open (surface) mines; waste-disposal establishments.

These activities (soil sealing) may cause irreversible, not or hardly correctable consequences, which means the decrease of multifunctional soil area, but does not mean that soil is lost and that it is a non-renewable natural resource.

**Soil functions** are all equally important, but the living organisms and their society

Table 3. Soil quality/health, as factors of sustainability

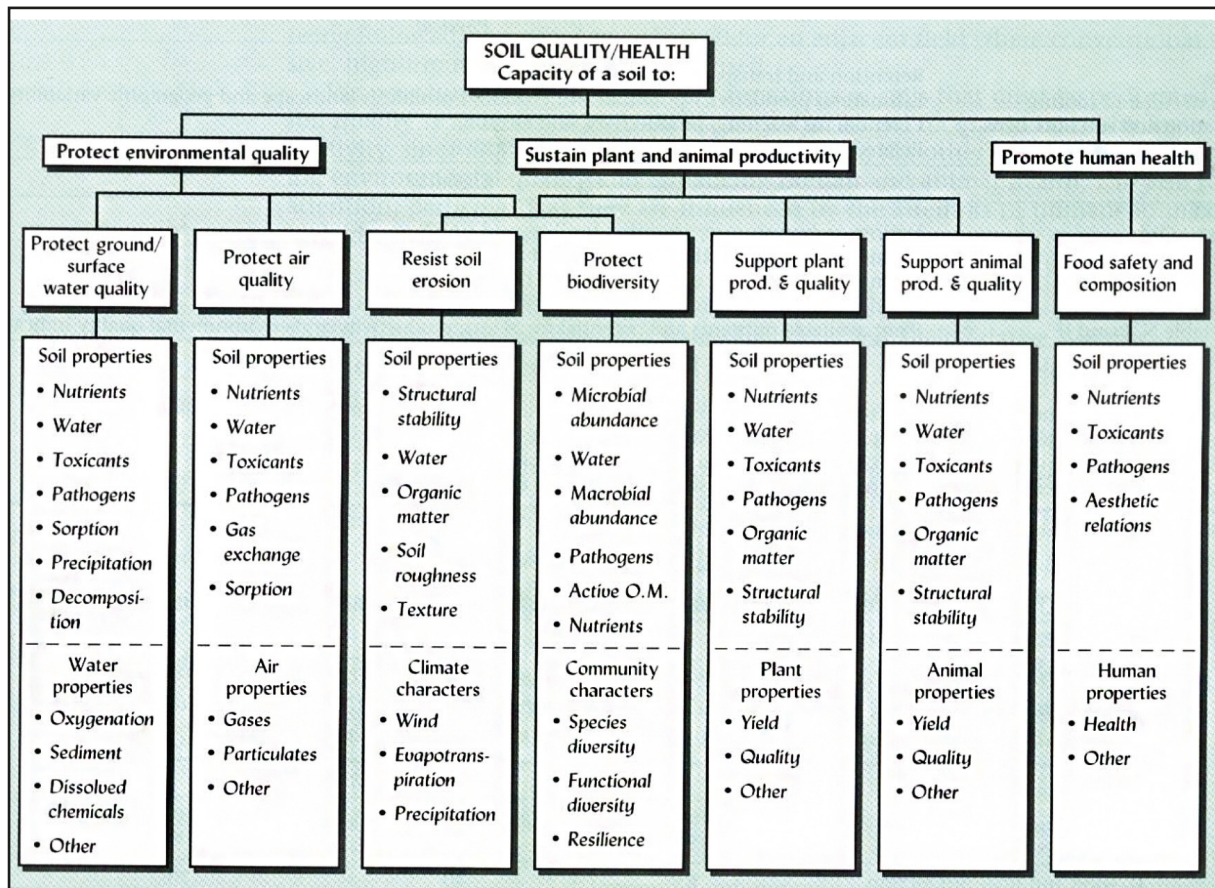
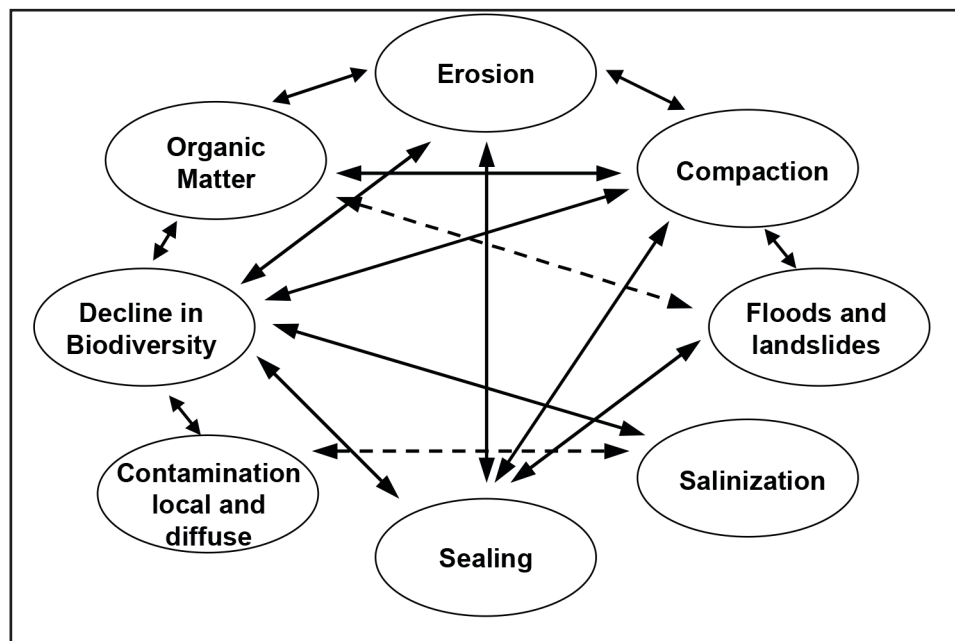


Figure 10. Land degradation problems in Europe



(even before the appearance of Man) natural conditions and socio-economic circumstances. In many cases the character (territorial and temporal variabilities, changeability–stability–controllability, method, efficiency) throughout the natural or human history, depending on the given

boundary conditions, limitations) of a certain function was not (properly or adequately) taken into consideration during the utilization of soil resources. In such cases the misguided management resulted in over-exploitation, decreasing the efficiency of one or more soil functions, and – above a certain limit – causing serious environmental deterioration (Várallyay, 1997, 2003, 2010a).

Soil multifunctionality doesn't mean that a given soil is *equally* able to fulfill all of the various (sometimes controversial) functions. It is the reason why „soil quality” is not a general term, but greatly *specific*, depending strongly on the main objective(s) and the *desired* function (Várallyay, 2000a). Rational and strict *priority setting* is crucially important in this respect! To help this priority setting Table 3 gives a list of soil properties and natural conditions for different „soil

quality” assessments for sustainable biomass production, for the control of environment, and for the promotion of human health (Lal, 2002, Várallyay, 2000a).

**Limiting factors of soil multifunctionality**

In the **European Soil Conservation Strategy** eight soil degeradation processes (threats to soil multifunctionality) get priority distinction (Figure 10). In the **Carpathian Basin** (especially on the Carpathian plains) the natural conditions are **relatively favourable for rainfed biomass production. In spite of this fact a considerable part of the soils are subject to various ecological constraints and unfavourable soil processes** (Láng et al., 1983, Szabolcs and Várallyay, 1978, Várallyay, 2006, Várallyay et al., 2010):

(a) **Soil degradation processes.** The main

Figure 11. Positive and negative consequences of anthropogenic interventions on soil functions and soil processes

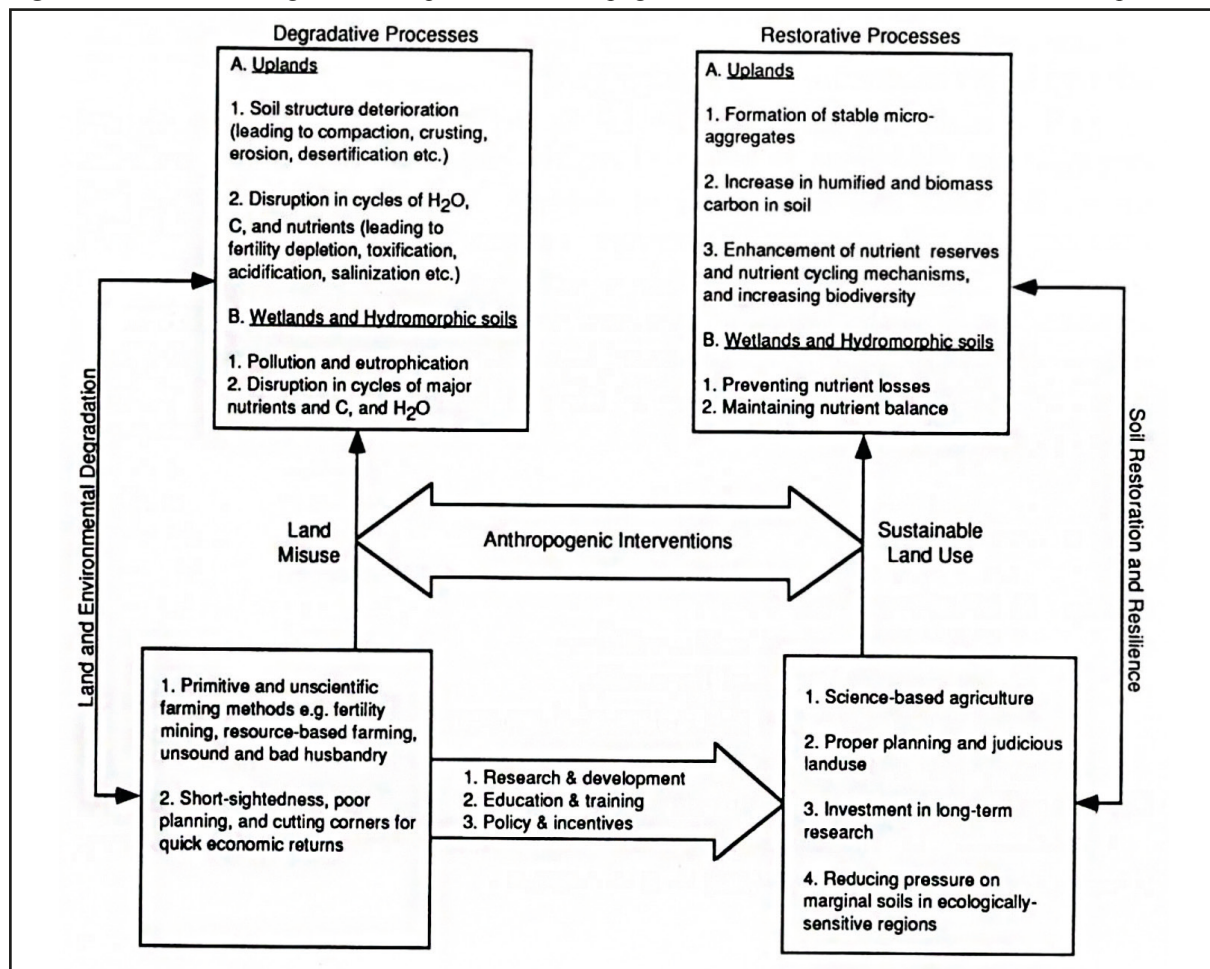
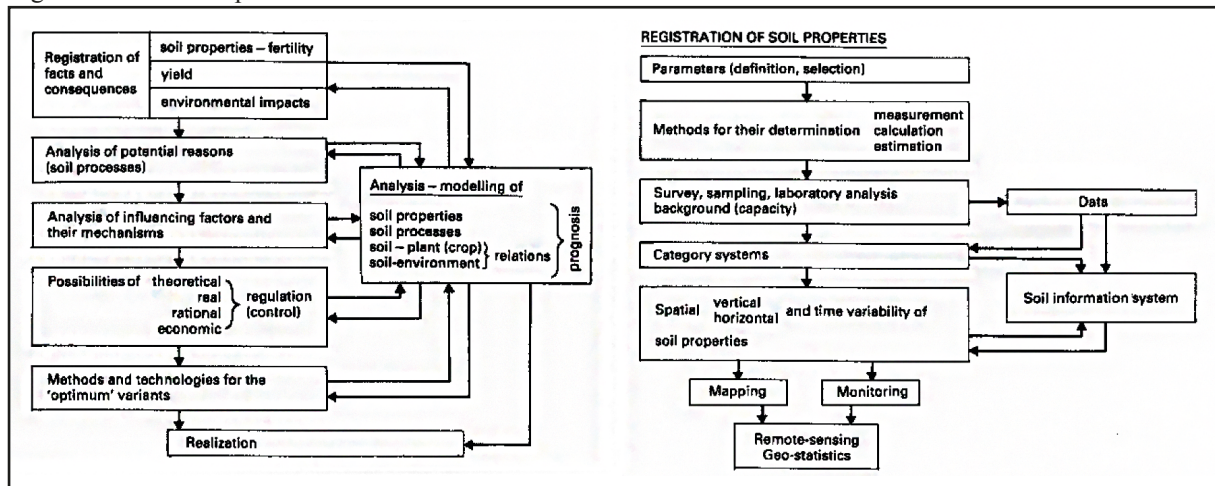


Figure 12. The conceptual model for the control of soil functions



soil degradation processes are: soil erosion by water or wind; soil acidification; salinization and/or alkalization; physical degradation (structure destruction, compaction); biological degradation; decrease in natural buffering capacity.

(b) **Extreme moisture regime:** the simultaneous hazard of waterlogging or over-moistening and drought sensitivity on large areas, sometimes in the same places within a short period.

(c) **Nutrient stresses.** The deficiency or accumulation and/or toxicity of one or more elements in the biogeochemical cycle.

(d) **Environmental pollution.** The accumulation or mobilization of various, potentially harmful (or even toxic) elements (or compounds) in the soil-water-plants-animals-human beings “food chain”.

**Control of soil functions**

The **multifunctionality** of soil is determined by the combined influences of soil properties, which are the results of soil processes: mass and energy regimes, abiotic and biotic transport and transformation, and their interactions. Any direct or indirect soil-related activity influences the land through these processes. Consequently, the **control of soil processes** is a great challenge and the

main task of soil science and soil management (Lal, 2002, Greenland and Szabolcs, 1993, Várallyay, 2000b). The positive and negative consequences of anthropogenic interventions on soil functions and soil processes are summarized in Figure 11. The conceptual model for the control of soil functions is shown in Figure 12 (Várallyay, 2000b).

The control of soil functions requires adequate **soil information**: exact, reliable, “detectable” (preferably measurable) and accurate, quantitative territorial data on well-defined soil and land properties, including the characterization of their spatial (vertical, horizontal) and temporal variabilities and pedotransfer functions; on the soil processes and biogeochemical cycles, including their determining and influencing factors and their mechanisms and on the actual and/or potential impacts of human activities. In Hungary a large amount of such information is available as a result of long-term observations, and various soil surveys, analytical and mapping activities conducted at the national (1:500,000), regional (1:100,000) farm (1:10,000–1:25,000) and field levels (1:5,000–1:10,000) over the past 60 years. A considerable part of these data were organized into a **GIS database** giving opportunities for an efficient control of soil processes and soil functions (Várallyay, 2000b, 2005).

## Conclusion

Soil is a four-phase, four-dimensional, polydisperse medium with three unique characteristics: conditionally renewable natural resource; resilience; multifunctionality. In addition to its primary function (fertility), the ability to produce multipurpose biomass (food, fodder, industrial raw material, alternative energy), modern society uses the other environmental (buffer, detoxication, biodiversity), technical (place and material for constructions, wellness) and even cultural (restore natural and human heritage) functions more and more intensely. Over- or irrational use sometimes are harmful threats to soil

and may result in non- or hardly correctable deterioration in soil multifunctionality, almost irreversible changes in ecosystems and – in acute cases – natural catastrophes with their tragical economical/ecological/environmental/social consequences. The rational and careful utilization and efficient **control of soil functions** is an inevitable task of *sustainability* for the enjoyable (but at least acceptable) life quality of present and future generations. Consequently, all efforts have to be taken – as priority – to maintain resilience, keep the renewable soil resources and create stable and sustainable conditions for their permanent renewal.

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## RESPONSE OF MAIZE AND WHEAT TO FERTDOLomite APPLICATION

Vlado KOVACEVIC<sup>1</sup> – Mirta RASTIJA<sup>1</sup> – Rezica SUDAR<sup>2</sup> – Dario ILJKIC<sup>1</sup> – Ivana VARGA<sup>1</sup>

<sup>1</sup> Faculty of Agriculture of University J. J. Strossmayer in Osijek, Kralja P. Svacica 1a, 31000 Osijek, Croatia; E-mail: vlado.kovacevic@pfos.hr

<sup>2</sup> Agricultural Institute Osijek, Juzno predgradje 17, 31000 Osijek, Croatia

**Abstract:** Granulated dolomite enriched with NPK (trade name fertdolomite: 24.0% CaO + 16.0% MgO + 3.0% N + 2.5% P<sub>2</sub>O<sub>5</sub> + 3.0% K<sub>2</sub>O) was applied (0, 5 t ha<sup>-1</sup>, 10 t ha<sup>-1</sup>, 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup> and 40 t ha<sup>-1</sup>) on standard fertilization. The field experiment was established in spring 2008. In the next years only standard fertilization was applied. In this study results of 3-year investigations (2011 -2013) were tested (rotation: maize – wheat – maize). Maize yields on the control were 10.84 and 10.04 t ha<sup>-1</sup>, for 2011 and 2013, respectively. Maize in the 2011 responded to fertdolomite up to quantity of 10 t ha<sup>-1</sup> by significant yield increases up to 9%, while by the rates 30 and 40 t ha<sup>-1</sup> yields were decreased up to 14%. However, in the 2013 effective (up to 17% yield increases) were 20 t ha<sup>-1</sup> and the higher doses. As affected by application of 20 t ha<sup>-1</sup> and the higher doses of fertdolomite four years ago, yields of wheat in 2012 were decreased compared to the control for 7% (7.47 and 6.96 t ha<sup>-1</sup>, respectively). Possible explanation of this phenomenon could be too high ears densities (871 vs. 982 ears square m<sup>-1</sup>) under the highest fertdolomite conditions and water deficit in tillering and stem elongation phases of wheat. Thousand grain weight, hectoliter mass and starch contents were independent on fertdolomite. However, protein-, wet gluten contents and sedimentation in wheat grain were considerably increased as affected by fertdolomite application as follows: 13.8% and 15.7% (protein), 33.5% and 38.1% (wet gluten), 46.5 and 58.1% (sedimentation value), for the control and the highest fertdolomite rate, respectively.

**Keywords:** maize, wheat, liming, fertdolomite, yield

### Introduction

Soil acidity is a major yield-limiting factor for crop production. The land area affected by acidity is estimated at 4 billion hectare, representing approximately 30 % of the total ice-free land area of the world (Sumner and Noble, 2003). Also, over half of the world population currently lives in regions dominated by acid soils (Yang et al., 2004).

Soil acidity is accompanied with some plant growth-limiting factors affecting physical, chemical, and biological properties of soil. Calcium (Ca), magnesium (Mg), and phosphorus (P) deficiencies or unavailability and aluminum (Al) toxicity are considered major chemical constraints that limit plant growth on acid soils. Disorders in activities of beneficial microorganisms, decomposition of organic matter, nutrient mineralization and immobilization, uptake and utilization of

nutrients by plants, are accompanied with soil acidity (Rengel, 2003; Kadar et al. 2007).

Liming is the most important and most effective practice to ameliorate soil acidity constraints for optimal crop production. Reduced soil acidity increases soil fertility by improvement of chemical, physical and biological properties of soil.

One of the benefits of liming acid soils are the increased utilization of P added by fertilizers because of reduced P fixation and increase of nitrogen (N) availability as affected by intensification of microbiological processes in soil (Rengel, 2003). Aim of this study was testing subsequent effects of fertdolomite application in spring 2008 on maize and wheat yields in 2011 - 2013 period. The results of the first three years of testing were shown in the previous study (Kovacevic et al., 2015).

## Material and methods

### *The field experiment, chemical and statistical analysis*

Granulated dolomite enriched with nitrogen, phosphorus and potassium (trade name fertdolomite – product of Petrokemija Fertilizer Factory in Kutina, Croatia: 24.0 % CaO + 16.0 % MgO + 3.0 % N + 2.5 % P<sub>2</sub>O<sub>5</sub> + 3.0 % K<sub>2</sub>O) was used for improvement of soil fertility on Drkulec Family Farm in Badljevina (municipality Pakrac, Pozega-Slavonian County). Fertdolomite was applied on standard fertilization in the amounts 0 (the control), 5 t ha<sup>-1</sup>, 10 t ha<sup>-1</sup>, 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup> and 40 t ha<sup>-1</sup> (Table 1). The field experiment was established at beginning of April 2008 in four replicates (basic plot 40 m<sup>2</sup>). In the next years only standard fertilization of the experiment was applied and subsequent effects of fertdolomite were tested.

The crop rotation on the experiment was as follows: maize (2008) - winter barley (2009) - maize (2010) – maize (2011) – winter wheat (2012) – maize (2013). The standard fertilizations of the experiment were 175 N + 50 P<sub>2</sub>O<sub>5</sub> + 50 K<sub>2</sub>O (maize), 120 N + 35 P<sub>2</sub>O<sub>5</sub> + 50 K<sub>2</sub>O (barley) and 175 N + 70 P<sub>2</sub>O<sub>5</sub> + 103 K<sub>2</sub>O (wheat). The results of the first three years of the experiment (2008 – 2010) were shown in the previous study (Kovacevic et al., 2015).

Farmyard in amount 35 t ha<sup>-1</sup> was ploughed in spring of 2012. Maize (the hybrid *Drava 404* developed on Agricultural Institute Osijek) was sown in the second half of April (2011 and 2013) by pneumatic sowing machine at planned plant densities (PPD) 70 391 plants ha<sup>-1</sup> and 68027 plants ha<sup>-1</sup>, for 2011 and 2013, respectively. Four internal rows from each basic plot were harvested manually at the first half of October. After harvest enumerated plants and mass of cob weighed by precise Kern electronic balance. Grain yields were calculated on 14% grain moisture basis.

Winter wheat (the cultivar *Srpanjka* developed on Agricultural Institute Osijek) was sown at middle part of October 2011 and harvested at beginning of July 2012. The ears of four quadrat areas (4 x 0.25 m<sup>2</sup>) were harvested by shears, enumerated, dried on open and thrashed by special combine for field experiments. Yields were calculated on 13% grain moisture basis.

Protein, starch, wet gluten and sedimentation value was determined by Near Infrared transmission spectroscopic method on Foss Tecator Infratec 1241 Grain Analyzer in Agrochemical laboratory of Agricultural Institute Osijek. The data were statistically analyzed by ANOVA and treatment means were compared using t-test and at 0.05 probability levels (LSD 5%).

Table 1. Quantities of the nutrients added by fertdolomite

Quantities of the nutrients added by fertdolomite (April 9, 2008) in Badljevina field experiment (four replicates, randomized block design, basic plot 40 m <sup>2</sup> )						
Fertdolomite* (t ha <sup>-1</sup> )		Added nutrients by fertdolomite (kg ha <sup>-1</sup> )				
		N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	CaO	MgO
a	0	0	0	0	0	0
b	5	150	125	150	1200	800
c	10	300	250	300	2400	1600
d	20	600	500	600	4800	3200
e	30	900	750	900	7200	4800
f	40	1200	1000	1200	9600	6400

\* added to the standard fertilization (SF); in the next years only SF of the experiment as follows (kg ha<sup>-1</sup>): 175 N + 50 P<sub>2</sub>O<sub>5</sub> + 50 K<sub>2</sub>O (maize), 175 N + 70 P<sub>2</sub>O<sub>5</sub> + 103 K<sub>2</sub>O (wheat).



*Soil and weather characteristics*

The surface soil layer up to 30 cm depth at starting of the experiment characterized by acid reaction (pH in 1nKCl 4.69), low levels of organic matter (2.12%) and plant available P (3.1 mg P<sub>2</sub>O<sub>5</sub> 100 g<sup>-1</sup>) determined by the AL-method (Egner et al., 1960). Hydrolitical acidity was 2.41 Cmol kg<sup>-1</sup>.

mainly because adequate and mainly good distributed precipitation. Both growing season characterized temperatures above the usual and these data are in harmony with the global warming (Table 2).

The growing season 2011/2012 was mainly favorable for winter wheat. Precipitation in the October-June period was 504 mm or

Table 2. The meteorological data for Daruvar (SHS, 2014)

Daruvar*: Precipitation and air-temperatures during maize and wheat growing seasons														
Year	Monthly precipitation (mm)							Monthly mean air-temperatures (°C)						
	The growing seasons of maize (2011 and 2013) and the long-term means (LTM): 1961-1990													
	Apr.	May	June	July	Aug	Sept	Σ	Apr.	May	June	July	Aug	Sept	X
2011	19	55	44	121	40	27	306	12.6	15.6	20.3	21.7	21.6	18.7	18.4
2013	69	82	55	61	54	106	427	12.5	15.8	19.2	22.1	21.7	17.3	18.1
61-90	77	86	99	86	91	65	504	11.0	15.7	18.9	20.6	19.7	16.1	17.0
The growing season of winter wheat (2011/2012) and the long-term means (LTM): 1961-1990														
	2011			2012										
	Oct.	Nov.	Dec.	Jan.	Febr.	March	April	May	June	Total	Mean			
mm	65	1	94	32	48	7	56	111	90	504				
°C	9.7	2.1	3.6	2.1	-2.7	8.4	11.9	15.9	21.4		8.0			
The long-term means (LTM): 1961-1990														
mm	64	82	66	55	49	58	77	86	99	635	7.9			
°C	10.9	5.8	1.4	-0.4	2.1	6.2	11.0	15.7	18.9					

\* 10 km from the experiment site in the N direction

The growing season 2011 was less favorable for maize growth mainly because of stress affected by combination of drought and high air-temperatures. In general, low yields of maize are in connection with water deficit and the high temperature, particularly in summer months (Markulj et al., 2010; Kovacevic et al., 2013). In the April-Sept. period of 2011 precipitation was 306 mm or about 40% lower than usual (LTM – Table 2). At the same period, mean air-temperature was 18.4 °C or the higher for 1.4 °C. This type of stress was the most effective in June and August because of precipitation quantities below 50% and the higher temperatures for 1.4 °C (June) and 1.1 °C (August). The growing season 2013 was more favorable for maize compared to 2011

for 21% lower than usual, while at the same period temperature was in level of the LTM. Deviations of the weather characteristic were drought periods in November and March (total 8 mm or only 6% of LTM), considerable colder November and February, as well as the higher temperatures in December and January (Table 2). In general, moderate lower precipitation, particularly in autumn / winter period and mild winter are favorable weather conditions for winter wheat (Marijanovic et al., 2010; Pepo and Kovacevic, 2011).

## Results and discussion

In two tested growing seasons, response of maize to fertdolomite were specific (Table 3),

Table 3. Impacts of fertdolomite application on maize properties

Impacts of fertdolomite * (Badljevin, April 9, 2008) on maize (the hybrid <i>Drava 404</i> )										
Fert-dolomite (t ha <sup>-1</sup> )		Plant density (PD) realization (PDR in % of planned PD*), unfertile plants (UP), grain moisture (GM) at harvest and grain yield (on 14% GM basis) of maize								
		The growing season 2011				The growing season 2013				
		Percent			Yield t ha <sup>-1</sup>	Percent			Yield t ha <sup>-1</sup>	
PDR	UP	GM	PDR	UP		GM				
a	0	95.5	15.8	22.7	10.84	89.6	5.9	28.2	10.04	
b	5	95.5	11.6	23.0	11.85	90.0	3.3	28.8	10.31	
c	10	95.5	26.1	23.4	11.66	89.6	6.1	29.0	10.65	
d	20	96.1	26.0	24.0	10.70	89.6	4.2	27.3	11.29	
e	30	96.5	35.0	23.5	9.50	93.0	4.4	27.0	11.68	
f	40	96.5	34.7	23.8	9.36	91.8	2.5	27.4	11.78	
Average		95.9	24.9	23.4	10.65	90.6	4.4	27.9	10.96	
LSD 5%				ns	0.70	LSD 5%			ns	0.80
Planned PD (100% PDR): 73261 plants ha <sup>-1</sup>						Planned PD (100% PDR): 68027 plants ha <sup>-1</sup>				
* composition: 24.0 % CaO + 16.0 % MgO + 3.0 % N + 2.5 % P <sub>2</sub> O <sub>5</sub> + 3.0 % K <sub>2</sub> O										

Table 4. Impacts of fertdolomite application (spring 2008) on winter wheat (the cultivar *Srpanjka*) status (the growing season 2011/2012)

Winter wheat status (2012) following fertdolomite (Fertd.) application (spring 2008): grain yield, ear number in unit of area, thousand grains weight (TGW), hectoliter mass (HM) and grain quality parameters (proteins, starch, moist gluten and sedimentation value contents)								
Fertd. t ha <sup>-1</sup>	Yield t ha <sup>-1</sup>	Ears per sqm <sup>-1</sup>	TGW g	HM kg	Grain quality parameters			
					Protein %	Starch %	Wet gluten %	Sedimentation value (mL)
0	7.47	871	30.5	77.3	13.8	65.4	33.5	46.5
5	7.77	934	31.4	78.0	13.4	66.2	32.5	45.0
10	7.41	927	30.1	77.1	14.9	64.6	36.1	54.5
20	7.01	956	29.3	76.6	15.2	64.5	37.1	55.0
30	7.06	982	30.6	76.8	15.5	64.1	37.7	56.6
40	6.96	977	31.1	77.1	15.7	63.9	38.1	58.1
Mean	7.28	941	30.5	77.2	14.8	64.8	35.8	52.6
LSD 5%	0.38	65	ns	ns	0.8	1.3	2.5	3.1

probably because of weather characteristic differences (Table 2). Maize in the 2011 responded positively to fertdolomite up to applied 10 t ha<sup>-1</sup> by significant yield increases up to 9%, while by the rates 30 and 40 t ha<sup>-1</sup> yields were decreased up to 14%. Explanation of this phenomenon could be in considerable increases of the unfertile plants, probably by the higher plant densities and more exposure to drought in flowering as affected

by observed about week earlier starting of flowering stage compared to plants on the control. The second possible explanation is restricted water supplies by precipitation in the first half of growing period (April - June 118 mm or 45% compared to the LTM) and following this more concentrated soil solution as affected by the very high applied fertdolomite doses. However, in the 2013 non-significant differences of yield between the

Table 5. Survey results of the first three years (2008 – 2010) of the field experiment (Kovacevic et al., 2015)

Impacts of fertdolomite * application (spring 2008) added to standard mineral fertilization on maize and barley: grain moisture at harvest (GM), grain yield, ears density in square m, P and K status in leaves (the ear-leaves at silking stage) and grain (maturity stage)						
	Maize (2008)		Spring barley (2009)		Maize (2010)	
Fertdolomite* t ha <sup>-1</sup>	GM %	Yield t ha <sup>-1</sup>	Ears (E) E m <sup>2</sup>	Yield t ha <sup>-1</sup>	GM %	Yield t ha <sup>-1</sup>
0	29.2	13.59	573	4.22	25.3	12.38
5	29.0	13.66	665	5.14	25.0	13.60
10	29.3	13.44	629	4.81	25.5	13.90
20	29.2	13.30	633	4.54	25.8	13.59
30	29.4	13.32	646	4.52	25.9	13.30
40	29.4	12.69	653	4.53	26.0	12.57
Mean	29.3	13.33	633	4.62	25.6	13.22
LSD 5%	ns	0.42	49	0.38	ns	0.40
LSD 1%		0.57	68	0.52		0.56

\* composition of fertdolomite: 24.0 % CaO + 16.0 % MgO + 3.0 % N + 2.5 % P<sub>2</sub>O<sub>5</sub> + 3.0 % K<sub>2</sub>O fertilization: 175 N + 50 P<sub>2</sub>O<sub>5</sub> + 50 K<sub>2</sub>O kg ha<sup>-1</sup> (maize), 120 N + 35 P<sub>2</sub>O<sub>5</sub> + 50 K<sub>2</sub>O kg ha<sup>-1</sup> (barley)

control and two the lower doses (5 and 10 t ha<sup>-1</sup>) were found and by applied the higher rates yields were increased up to 17% compared to the control. In the both growing season, fertdolomite did not impacts on grain moisture at harvest (Table 3).

As affected by application of 20 t ha<sup>-1</sup> and the higher fertdolomite doses four years ago, yields of wheat in 2012 were decreased up to 7%, while by applied the lower rates yields were in level of the control. Possible explanation could be too high ears densities and water deficit in tillering and stem elongation phases of wheat plants under the higher fertdolomite conditions.

Thousand grain weight and hectoliter mass and starch contents were independent on fertdolomite. However, protein-, wet gluten contents and sedimentation value in wheat grain were considerably increased as affected by fertdolomite application as follows: 13.8% and 15.7% (protein), 33.5% and 38.1% (wet gluten), 46.5 and 58.1% (sedimentation value), for the control and the highest fertolomite rate, respectively (Table 4).

Grain yield and grain protein concentration

are important traits affecting the economic value of common wheat. There is an inverse relationship between grain yield and protein content which is corroborated by our study. Wheat proteins and wet gluten is a primary quality component that influences the most of wheat grain baking quality characteristics and applied fertilization affected positively to these properties.

Kovacevic et al. (2015) reported about results of the first three years of the experiment (2008 -2010). In 2008, non-significant differences of maize yields among the control and limed treatments until 30 t ha<sup>-1</sup> were found, but over liming by 40 t ha<sup>-1</sup> resulted by yield decreases for 7% (13.59 and 12.69 t ha<sup>-1</sup>, respectively), mainly due to reduction of plant density. However, in 2010, as affected by fertdolomite yields of maize were increased until 12% compared to the control (12.38 and 13.90 t ha<sup>-1</sup>, respectively). With that regard, non-significant yield differences of limed treatments from level between 5 and 30 t ha<sup>-1</sup> were found, while by the highest step (over liming) yield was decreased to level of the control. Even the lowest rates of fertdolomite in amount of 5 t ha<sup>-1</sup> was adequate for considerable yield

increase of spring barley in 2009 for 22% compared to the control (4.22 and 5.14 t ha<sup>-1</sup>, respectively), but by the highest treatment yield was reduced to level of the control.

By the other experiments realized in the last 15 years in the northern Croatia (Antunovic, 2008; Jurkovic et al., 2008; Mesic et al., 2009; Kovacevic and Rastija, 2010; Andric et al., 2012; Kovacevic et al., 2006; Kovacevic and Loncaric, 2014) and the northern Bosnia (Markovic et al., 2008; Komljenovic et al., 2013) were found also mainly positive effects of liming on field crops yield. With that regard, as in our study, considerable effects of the growing season characteristics (the factor "year") on field crops yield and liming efficiency were found.

### Conclusions

Based on the results of our study and majority studies realized in Croatia, liming had positive effects on field crops yield with considerable influences of growing season impacts of liming efficiency, mainly due to weather characteristics. Maize in the 2011 responded to fertdolomite up to quantity of 10 t ha<sup>-1</sup> by

significant yield increases up to 9%, while by the rates 30 and 40 t ha<sup>-1</sup> yields were decreased up to 14%. However, in the 2013 effective (up to 17% yield increases) were 20 t ha<sup>-1</sup> and the higher doses. As affected by application of 20 t ha<sup>-1</sup> and the higher doses of fertdolomite four years ago, yields of wheat in 2012 were decreased compared to the control for 7%, probably due to too high ears densities under the highest fertdolomite conditions and water deficit in tillering and stem elongation phases of wheat.

However, fertdolomite positively affected on backing quality parameters of wheat grain as follows: 13.8% and 15.7% (protein), 33.5% and 38.1% (wet gluten), 46.5 and 58.1% (sedimentation value), for the control and the highest fertdolomite rate, respectively.

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## RESPONSE OF MAIZE TO LIMING AND PHOSPHORUS FERTILIZATION WITH EMPHASIS ON WEATHER PROPERTIES EFFECTS

Ilija KOMLJENOVIC<sup>1</sup> – Mihajlo MARKOVIC<sup>1</sup> – Goran DJURASINOVIC<sup>2</sup> – Vlado KOVACEVIC<sup>3</sup>

<sup>1</sup> Faculty of Agriculture, University of Banja Luka, Sq. Vojvoda Bojovic bb, 78000 Banja Luka, Republic Srpska (RS), Bosnia and Herzegovina, E-mail: ilijakom@teol.net

<sup>2</sup> Agricultural Institute of Republic of RS, Knjaza Milosa 18, 78000 Banja Luka, RS, Bosnia and Herzegovina

<sup>3</sup> Faculty of Agriculture of University J. J. Strossmayer in Osijek, Kralja P. Svacica 1d, 31000 Osijek, Croatia

**Abstract:** The field experiment of liming (0 and 10 t ha<sup>-1</sup> of powdered hydrated lime) and phosphorus (P) fertilization (monoammonium phosphate or MAP: 12% N + 52% P<sub>2</sub>O<sub>5</sub>) started in autumn 2008 on acid soil of Laktasi municipality (Republic of Srpska, Bosnia and Herzegovina). Three doses of P (0, 500, 1000 and 1500 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) on ordinary fertilization were applied. The experiment was conducted in four replicates. Basic plots of liming and P fertilization were 640 m<sup>2</sup> and 40 m<sup>2</sup>, respectively. Maize was grown in monoculture. Under drought stress of 2011 and 2012 yields were considerably lower (mean 3.86 t ha<sup>-1</sup>) than in the remaining two years (mean 9.20 t ha<sup>-1</sup>). As affected by drought and high air-temperature, particularly in August, the 2012 growing season was especially unfavorable for maize growth because mean yield in the experiment was only 2.06 t ha<sup>-1</sup> or 22% of yield realized in the 2010 growing season. As affected by liming yield of maize was increased by 31% (4-year mean), while P effect was considerably lower (6.14 and 6.65 t ha<sup>-1</sup>, for the control and average of ameliorative P treatments, respectively).

**Keywords:** liming, phosphorus fertilization, maize, grain yield. Lijevece polje area

### Introduction

Bosnia and Herzegovina (B&H) has 2.159 million hectares of agricultural area. Arable land covers 1.006 million hectares or about 46% of agricultural land and 0.53 million hectares is covered by temporary crops. Maize and wheat are the most frequently grown field crops on arable land of B&H. Annual harvested area (5-years average 2008-2012) of maize was 194 836 ha and for wheat 61 180 ha and both crops covered close to 50% of temporary crops area (FAO, 2013).

Grain yields of the field crops in B&H (averages 2008-2012) are mainly low (4.24 t ha<sup>-1</sup> for maize and 3.50 t ha<sup>-1</sup> for wheat) with considerable differences of yield among the years (from 2.75 to 4.92 t ha<sup>-1</sup> and from 2.66 to 3.78 t ha<sup>-1</sup>, for maize and wheat, respectively: FAO, 2013).

Acid reaction and nutritional unbalances, mainly low levels of plant available phosphorus (P) as well as unfavorable physical properties are oft limiting factor of soil fertility in B&H (Okiljevic et al., 1997, Resulovic and

Custovic, 2002). These characteristics of soils in combination with very low consumption of mineral fertilizers (19.5 kg ha<sup>-1</sup> on 1022000 ha: The World Bank, 2012) are main factors of low yields of field crops in B&H.

Aim of this study was testing the maize response to P fertilization and liming in Lijevece Polje area (RS, B&H) with emphasis on the growing season ("year") effects, while response of maize in consecutive four years of testing were shown in two previous studies (Komljenovic et al., 2013, 2015).

### Materials and methods

#### *General description of the Lijevece Polje area*

Lijevece polje (Lijevece field) is an area in the northern part of B&H, encompassing the Gradiska, Laktasi and Srbac municipalities in the entity of Republic of Srpska (RS). It is lowland area in the lower flow of the Vrbas river, extending from the Sava river to the north and the mountains Prosara to the west, Motajica to the east and Kozara to the

southwest. Climate of this area is moderate continental. In general, soil is more fertile compared to the majority of agricultural areas in the country, although serious problems of aluminum toxicity were sporadically found (Okiljevic, 1982; Kovacevic et al., 1988).

#### Description of the field experiment area

This research was carried out on the Djurasinovic Family Farm in Mahovljani (Laktasi municipality, RS, B&H) during four consecutive growing seasons, from 2009 to 2012, on pseudogley soil ( $\text{pH}_{\text{InKCl}} = 4.28$ ) low supplied with plant available P and rich in potassium (K). The treatments included liming and P fertilization. P distribution as monoammonium phosphate (MAP: 12% N + 52%  $\text{P}_2\text{O}_5$ ) was conducted on November 10<sup>th</sup>, 2008 before ploughing. The rates of P on basic fertilization (160 N + 75  $\text{P}_2\text{O}_5$  + 75  $\text{K}_2\text{O}$  kg  $\text{ha}^{-1}$ ) were as follows: 0, 500, 1000 and 1500 kg  $\text{P}_2\text{O}_5$   $\text{ha}^{-1}$ . Immediately after P fertilization the experiment plot was ploughed up to 30 cm in depth. Liming of the experiment by 10 t  $\text{ha}^{-1}$  of powdered hydrated lime (73% CaO + 2-3% MgO + 21% of bound water) was made on November 16<sup>th</sup>, 2008. The experiment was conducted in four replicates (basic plots 40  $\text{m}^2$  and 640  $\text{m}^2$  for P and liming treatments, respectively). Maize (the hybrid NS444) was grown in monoculture. Detailed information regarding crop management practice, weather characteristics, statistical analysis, grain yield, grain moisture, protein-, starch- and oil-determinations, were shown in the previous study (Komljenovic et al., 2013).

#### Collection of the weather data and calculation of water balance

Statistical Yearbook of RS (SY, 2013), Hydrometeorological Service of RS in Banja Luka (HSRS, 2012) and TuTiempo (2012) and Saric et al., (1996) were the sources of meteorological data. Calculations of potential evapotranspiration (PET), actual evapotranspiration (AEV), water deficit (WD) and WS (water surplus) were made according

to Thornthwaite and Mather (1955) and (1957) as follows:

$$PET = 16 \left( \frac{10T}{I} \right)^a \quad (1)$$

$$I = \sum_1^{12} i \quad (2)$$

$$i = \left( \frac{T}{5} \right)^{1.514} \quad (3)$$

$$a = 0.00000075 x I^3 - 0.0000771 x I^2 + 0.01792 x I + 0.49239 \quad (4)$$

Where:

- PET = mean monthly potential evapotranspiration (mm)
- T = mean monthly air temperature ( $^{\circ}\text{C}$ )
- I = annual heat index;
- i = monthly heat index

#### **Results and discussion**

Average grain yield of maize in the experiment was considerable different among the years, ranging from 2.06 to 9.38 t  $\text{ha}^{-1}$ . Under drought stress of 2011 and 2012 yields were considerably lower (mean 3.86 t  $\text{ha}^{-1}$ ) than in the remaining two years (mean 9.20 t  $\text{ha}^{-1}$ ). As affected by drought and high air-temperature, particularly in August, the 2012 growing season was especially unfavorable because mean yield in the experiment was only 2.06 t  $\text{ha}^{-1}$  or 22% of yield realized in the best 2010 growing season (Table 1). Low yield in the experiment was accompanied by a very high contribution (about 50%) of barren plants (Komljenovic et al., 2015) due to drought and high temperatures at flowering stage.

As affected by liming yield of maize was increased by 31% (4-year mean), with considerable differences among years (from 18% in 2010 to 47% in 2011). Ameliorative P fertilization impact on maize yield was considerably lower compared to liming effect because yields were increased in three years



Table 1. Response of maize to liming and P fertilization

Liming (the factor A) by the hydrated lime (A1 = 0, A2 = 10 t ha <sup>-1</sup> ) and ameliorative phosphorus (P <sub>2</sub> O <sub>5</sub> kg ha <sup>-1</sup> : B1= 0, B2 = 500, B3 = 1000, B4 = 1500 ) fertilization (the factor B) in autumn 2008: impacts on grain yield of maize in four consecutive growing seasons 2009 -2012 (Komljenovic et al., 2013, 2015)												
	2009			2010			2011			2012		
	A1	A2	x B	A1	A2	x B	A1	A2	x B	A1	A2	x B
	Grain yield (t ha <sup>-1</sup> )			Grain yield (t ha <sup>-1</sup> )			Grain yield (t ha <sup>-1</sup> )			Grain yield (t ha <sup>-1</sup> )		
B1	7.30	10.07	8.68	7.97	9.92	8.94	4.40	5.53	4.97	1.82	2.08	1.95
B2	7.37	10.65	9.01	8.66	10.28	9.47	4.59	7.19	5.89	1.98	2.24	2.11
B3	7.61	10.66	9.13	9.23	10.24	9.73	4.60	7.09	5.85	1.85	2.36	2.11
B4	7.82	10.60	9.21	8.55	10.17	9.36	4.68	7.06	5.87	1.67	2.49	2.08
x A	7.52	10.49	<b>9.00</b>	8.60	10.15	<b>9.38</b>	4.57	6.72	<b>5.65</b>	1.83	2.29	<b>2.06</b>
A effect		+39%			+18%			+47%			+25%	
B effect			+6%			+9%			+19%			ns
	A	B	AB	A	B	AB	A	B	AB	A	B	AB
P 0.05	0.81	0.43	ns	0.32	0.33	ns	0.45	0.34	0.49	0.17	ns	ns
P 0.01	1.49	ns	ns	0.59	0.45		0.83	0.47	0.67	0.30		

from 6% in 2009 to 19% in 2011, while in 2012 differences of yield among P treatments were non-significant. By comparison of yield realized by three ameliorative P rates and the control, P effect on maize yield was only 8% (4-year averages: 6.14 and 6.65 t ha<sup>-1</sup>, respectively). With exception of the first year of testing, for significant increase of maize yield was adequate the lowest rate of applied P in amount 500 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (Table 1).

Positive effects of either liming or ameliorative P fertilization on yields of field crops in B&H, as well as in the neighboring Croatia, were found by the earlier studies (Kovacevic et al., 2006, 2014; Antunovic, 2008; Markovic et al., 2008; Mesic et al., 2010; Komljenovic et al., 2010; Kovacevic and Rastija, 2010; Andric et al., 2012).

Precipitation quantity and distribution, as well as temperature regime during growing season, considerably affected the yields of crops under field conditions (Shaw, 1988, Kovacevic et al., 2013). With that regard, in our experiment two growing season were favorable for maize growth (2009 and particularly 2010), while remaining two were less favorable, particularly the 2012 growing season (Table 2).

Precipitation in May-September period was

in three growing seasons below LTM, while only in 2010 precipitation was close to 50% higher. In accordance with the trend of global warming (Komljenovic et al., 2014), average temperatures in the mentioned period were in all four growing seasons higher than usual, particularly in July and August (Table 2).

The 2009 growing season was characterized in total by moderate and considerable deviations of monthly precipitation compared to usual (LTM). However, majority precipitation was in June-August (335 mm or close to 70% of total precipitation in May-September period) when maize has very high needs for water. Mean air-temperatures in May-Sept. was 19.2°C or 2.3°C higher than usual. July and August were warmer compared to LTM for 2.7°C and 3.1°C, respectively.

The growing season 2010 was especially favorable for maize because of adequate precipitation for maize growth in all stages of development and something lower temperatures.

The growing season 2011 was moderate in precipitation (May-Sept.: 310 mm or only 56% from LTM) with adequate amount only in July and considerable deficit in June, August and September (total 72 mm or only 25% from

Table 2. Precipitation and air-temperatures in Banja Luka (SY, 2013; HSRS, 2012; TuTiempo 2012)

The weather data (Banja Luka* Weather Bureau: LTM = long-term averages 1961-1990)										
Year	Jan.-April	May	June	July	Aug.	Sept.	Oct.	May-Oct.		
Precipitation (mm)										
2009	235	49	153	44	138	33	73	489		
2010	419	148	235	66	87	196	84	816		
2011	153	63	37	113	9	26	62	310		
2012	244	168	70	53	2	92	88	473		
LTM**	298	98	111	95	93	82	72	551		
Mean air-temperatures (°C)										
2009	5.9	18.9	20.0	23.3	22.8	18.6	11.4	19.2		
2010	5.5	16.5	20.3	23.1	21.8	15.7	9.4	17.8		
2011	5.9	16.0	21.2	23.1	23.7	20.2	11.0	19.2		
2012	5.3	16.1	23.0	25.2	24.4	18.9	12.5	20.0		
LTM	4.9	15.6	18.9	20.6	19.7	15.9	10.8	16.9		
Absolute and average maximum air-temperatures (°C), PET, AET, WD and WS (mm) in Banja Luka**										
Year	Absolute maximal temp.			Average maximal temp.			PET	AET	WD	WS
	June	July	August	June	July	August	June –August (sum)			
2009	33	37	38	29.9	25.1	29.5	366.6	326.4	40.2	0.0
2010	34	38	35	27.9	24.9	27.7	361.0	329.1	32.0	119.0
2011	33	37	36	28.4	27.0	30.2	379.6	213.2	166.4	0.0
2012	35	40	37	31.1	28.4	32.6	414.7	219.3	195.3	0.0
* air-distance from the experiment site: Banja Luka = about 20 km in S direction										
** PET = potential (PET) and actual (AET) evapotranspiration, water deficit (WD) and water surplus (WS)										

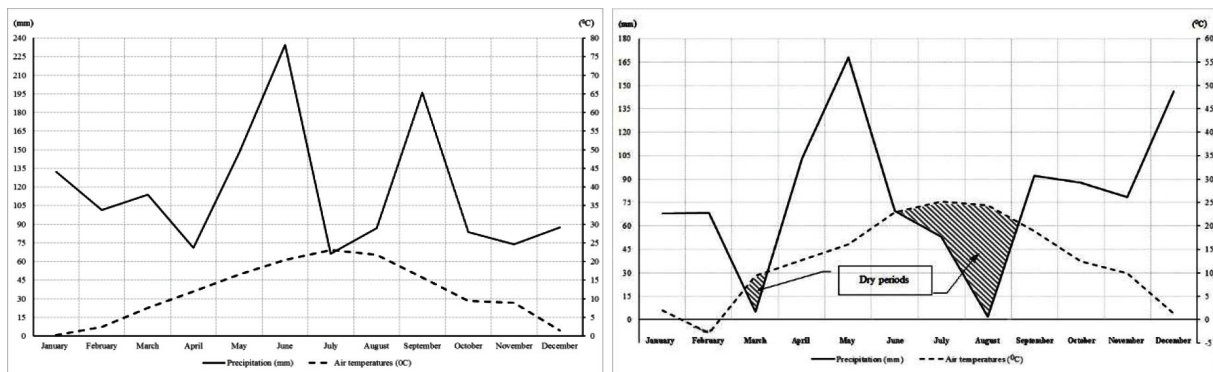
LTM). Aggravating circumstance was low reserve of water in soil before sowing (153 mm precipitation in Jan.-April or about 50% lower than usual). Water deficit in August in combination with average temperature 4.0°C higher than LTM are the most responsible for low yield of maize in the experiment. The 2012 growing season was very unfavorable for maize growth. Weather in Banja Luka during 2012 was characterized by water deficit in August (2 mm precipitation) accompanied by mean air-temperature of 24.4°C or 3.1°C higher compared to the long-term mean. Also, air-temperature in July was 25.2°C or 3.4°C higher than average. Absolute maximum air-temperature increased to enormous level of 40°C and average maximum temperature to 32.6°C (Table 2). Observing a long-term average for Banja Luka area (2009-2012), the state of soil moisture, PET and AET, for the period June-August, the most evident water surplus was in 2010. On the other hand, in case of deficiency of moisture, as opposed to the year 2010, in the analyzed 2012, there was

a very significant deficit of moisture (WD) and significant difference between PET and AET. This led to a very dry period and the lack of moisture in the soil (Table 2).

As affected by these stress conditions, maize was dried at the end of August without green color of leaves.

Maize covers about 26% (status 2012 according FAO, 2013) of arable land in the countries of the region. It is main field crop in Serbia (37.2%), Romania (28.7%), Hungary (26.8%), Croatia (21.5%) and B&H (19.4%). For this reason, unfavorable weather conditions for maize, for example in 2012, are a considerable negative factor for agriculture and economy development in these countries. As affected by unfavorable weather conditions in 2012, yield reduction of maize in the countries of the region compared to normal 2010 was as follows: 53% in Serbia, 49% in Romania, 40% in B&H and 38% in Hungary and Croatia (Kovacevic et al., 2013). Based on FAO (2013), in level of the region covering

Figure 1. Climate diagram by Walter in Banja Luka for the period 2010 (left) and 2012 (right)



these five countries, maize yield reduction in 2012 was about 47% compared to 2010 (2.89 and 5.41 t ha<sup>-1</sup>, on the harvested areas 5.21 and 6.14 million ha, respectively).

Based on Walter's climate diagram for Banja Luka area for the two tested periods (2010 and 2012), it can be clearly noted that in 2010 during mid-June to late August there was enough rainfall and even surplus, in contrast to the same period in 2012 with extreme drought, (Fig. 1). This is the most critical period for flowering, fertilization and grain filling of maize. Lack of moisture during this period caused a significant drop in the yield of corn. This suggests that irrigation of cultivated crops, with their growing period coinciding with the mentioned period of the lack of water, becomes necessary in the agricultural production within the area (Komljenovic et al., 2014) Irrigation in the critical periods is a direct management practice for overcoming drought stress in plants (Josipovic et al., 2012). Indirect managements for alleviation of this stress is ploughing for spring crops in autumn/winter period instead in spring together with incorporation of adequate quantities of mineral fertilizers, weed control etc.

## Conclusion

In our study, precipitation quantity and temperature regime ("year effects") were the influencing factors on maize yield under environmental conditions of northern Bosnia. The second-ranked tested factor was liming, while P fertilization had mainly moderate impact on yield. Both liming and P fertilization effects were in high interaction with weather characteristics, particularly in two summer months July and August. Irrigation is a direct management practice for overcoming drought stress in arable crops. Also, alleviation of negative effects of drought is possible by adequate soil tillage and adequate fertilization and weed control.

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## PLANT GENES AS SOURCES TO IMPROVE PATHOGEN RESISTANCE OF CULTIVATED PLANTS

Zsófia TÓTH – Erzsébet KISS

Szent István University, Institute of Genetics and Biotechnology, 1. Páter Károly Street, 2100, Gödöllő, Hungary  
E-mail: Toth.Zsofia@mkk.szie.hu, Kiss.Erzsebet@mkk.szie.hu

**Abstract:** Diseases cause large problem in agriculture that the growers usually try to overcome by chemical control. Unfortunately, the amount of applied chemicals increases in every year all over the world. Since the chemicals have numerous negative side-effects, the alternative techniques are welcome in crop production. Plenty of studies demonstrated that resistance genes regulate plant defense response to pathogen attack and infection. Beside the induced expression of resistance genes the disease responsiveness may also depend on plant susceptibility genes, presence in host of which is required for success of invasion. Breeding plant varieties integrating resistance genes or inhibiting the function of susceptibility genes significantly increase the disease resistance. In this manner the amount of pesticides applied for pathogen control can be reduced and crop production may turn into a much more environmentally friendly process.

**Keywords:** pathogen response, signaling, resistance genes, susceptibility genes, plant breeding

### Introduction

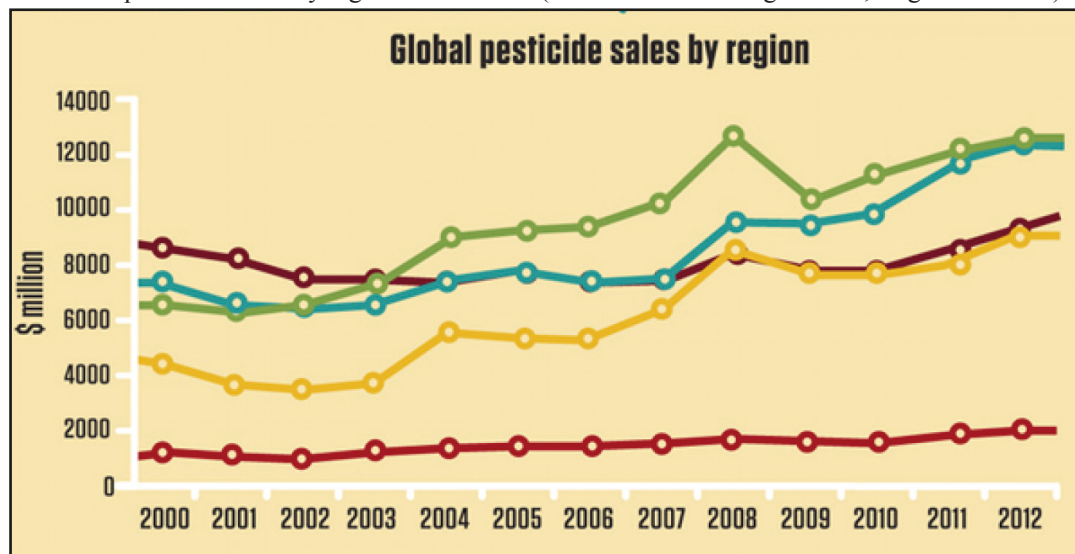
Although numerous studies quest the genetic and molecular background of diseases and already a lot of results have been born, the pathogenic organisms still cause serious problems in agriculture. The growers apply chemicals on their fields to control the invasion of pathogens. The fairly effective - but because of the necessity of repeated spraying - very expensive chemicals require skilled labour with stringent regulatory restrictions. Although this process significantly increases the prime cost of crop production, the amount of chemicals applied to control pest increases every year (Figure 1). The world used approximately 5.2 billion pounds of pesticides per year in the last decade, but it still raised in the subsequent years (FAOSTAT). Mostly, herbicides are used all over the world, but the insecticide and fungicide consumption is also high in some regions due the impact of climate conditions, such as high humidity. Beside the primary high cost, the application of chemicals has high risk of potentially harmful impact on the environment, ecosystems and consumers. The toxicity of some chemicals increases the number of morbidity cases in all over the world. The human health hazards range from acute dangers to serious chronic

diseases, such as cancer, endocrine disruption. Therefore studies aspiring development of new alternative techniques to control diseases are exceptionally conducive. Many chemical-free opportunities are to limit disease spread, such as agrotechnical methods, integrated pest management or application of resistant/tolerant varieties for cultivation. These techniques are excellent scopes to grow crops environmentally friendly, and together may be sufficient to fight against diseases without yield loss. Breeding new varieties with integration of resistance genes is an admitted technique using nowadays the marker assisted selection (MAS). By MAS the breeding period may be limited to two years, which is a large progress compared to the traditional techniques. Studies discovering new genes, which relate to defense or promote immune response support the new plant breeding purposes.

### Regulation of plant defense during host-pathogen interaction

Plants are subjects of numerous challenges during their lifecycle. Since they are immobile organisms, cannot escape from attackers, the plants were forced to develop complex defense system to survive at a higher rate. During the long host-pathogen co-evolution, plants configured specific prevention

Figure 1. Global pesticides sales by region of the world (Source: The Washington Post, August 18, 2013).



mechanisms, such as strengthened cell wall, or production of antimicrobial compounds. Once the pathogen passes these tricks, and enters the plant cell, the plant recognizes the pathogen-secreted effector proteins by its 'receptors'. These 'receptors' are encoded by resistance (*R*) genes and while the effectors proteins by the pathogenic *avr* genes. The mutual recognition is called the 'gene-for-gene' interaction, first described in 1942 (Flor, 1942, 1971). If these two proteins interact, the reaction induces a complex signaling cascade which may end with systemic acquired resistance (SAR), or with programmed cell death, the hypersensitive reaction (HR) (Király et al., 1972; Fodor et al., 1997). Both responds are mediated via salicylic acid signaling and significantly limit the pathogen growth; HR totally inhibits infection. This type of immune response is called the effector-triggered immunity (ETI), which based on the specific gene-for-gene interaction. If this specificity does not exist, the plant still may recognize its aggressor by the pathogen- or microbe-associated molecular pattern (PAMP, MAMP). The specific molecular pattern may be the bacterial flagellins, lipopolysaccharides, nucleic acids (e.g. viral and bacterial DNA/RNA), peptidoglycans, fungal chitins or glucans. These molecules can be perceived by pattern recognition receptors (PRR) of the

plant and the detection induces the PAMP-triggered immunity (PTI), a form of basal defense. During PTI process mainly those genes are activated, which are involved in the biosynthesis of antimicrobial compounds, promote pathogenic cell wall degradation, regulate plant cell wall fortification or stomatal closure. Some of the signaling components of PTI overlap with the ones of ETI, but somehow the PTI-triggered defense reaction is not as effective to control pathogen spread, as the HR or SAR is. Additionally to PTI the plant cell detects microbial compounds released by the injured microorganisms. For example cucumber hypocotyl recognized the  $\alpha$ -1,4-linked oligomers of galacturonic acid and oligo- $\beta$ -glucans released from damaged *Phytophthora megasperma* f. sp. *glycinea* cell wall triggering hydrogen-peroxide production (Svalheim and Robertsen, 1993). This phenomenon is called Damage Associated Molecular Pattern (DAMP), similar mark of pathogen presence as PAMP/MAMP and provokes DAMP-triggered immunity (DTI). The susceptibility of the plant to diseases is not always an obligatory result of host immunity failure. Earlier studies demonstrated that susceptibility of many plant species rather depends on host compatibility factors, than early response of PTI or *R* genes. Numerous genes are identified to play a role in advancing



pathogen proliferation, especially of biotrophic fungi, which require cooperation of host compatibility factors for their invasion. The genes impairing prepenetration requirements (enable the pathogen to enter the plant cell) or fulfilling postpenetration necessities are termed as susceptibility (*S*) genes.

### **Difference between necrotrophic and biotrophic pathogens**

Fungi and oomycetes produce spores, which germinate on plant surface and develop fungal hypha. The fungus may enter the plant cell through natural openings, or may punch the cell wall using their appressoria and form the haustoria for feeding and effector transmission (Yi and Valent, 2013). Early response to fungi may associate with papilla formation and cell death along with accumulation of H<sub>2</sub>O<sub>2</sub> (Huckelhoven et al., 1999). Contrarily the bacteria are unable to breach cell wall, therefore these organisms resort to the natural openings, such as wounds or stomata. Bacteria often form the type III or type IV secretion system to translocate their elicitor molecules, which may induce early response of plant defense (Ott et al., 2006; Klement et al., 2003; Bozsó et al., 2005) Based on lifestyle of the pathogen we discriminate necrotrophic and biotrophic organisms. The necrotrophic pathogens degrade their host and utilize nutrients from the rotten tissues. In contrast, the biotrophic pathogens survive only on live plants, therefore they are able to manipulate plant metabolism to retard senescence of the infected tissue ('green island' effect) (Bushnell and Allen, 1962). The hemibiotroph organisms are considered as necrotrophs, but have an initial biotrophic lifestyle of the early stage of infection. Especially the hemibiotroph *Colletotrichum graminicola* fungus induces green island effect, but it also globally accelerates senescence in aging maize leaves in order to utilize them as carbon sink (Behr et al., 2010). Since the lifestyle differs to a great extent, the immune response is also distinct

in many aspects between bio-, hemibio- and necrotrophs. For example the gene-for-gene interaction is assumed to occur only in biotrophs; in necrotrophs the receptor-like protein kinases (RLKs) mediated PTI induces the response (Llorente et al., 2005). The plant defense signaling components also differ between the infection styles; the salicylic acid (SA)-mediated signal transduction is active usually in biotroph attack, but the ethylene (ET)/jasmonic acid (JA) signals regulate on the effect of necrotroph infection mostly. The hydrogen-peroxide and other reactive oxygen intermediates (ROI) may also provide signaling function during the infection; these components are found to promote the HR, often called oxidative burst. The H<sub>2</sub>O<sub>2</sub> is the most stable ROI that may induce defense gene-expression, activate phytoalexin production during biotic stress. It was also reported that this molecule inhibit biotroph growth, but mostly benefit necrotroph invasion (Thordal-Christensen et al., 1997). However, the NADPH oxidase gene, *RBOHD* (for respiratory burst oxidase homolog D) was found to regulate HR in *Arabidopsis-Alternaria* pathosystem specifically in the infected single cells and not in neighboring ones (Pogany et al., 2009). Additionally externally applied H<sub>2</sub>O<sub>2</sub> inhibited replication of Tobacco mosaic virus (TMV), therefore early accumulation of ROI promotes resistance to TMV (Bacsó et al., 2011).

Mutation analysis is a prominent approach for identifying the components of the signaling pathways. Many recessive mutations result in a constitutive defense response, such as the *acd2* (accelerated cell death), *cim3* (constitutive immunity), *cpr1-1* (constitutive expressor of pathogenesis-related (*PR*) genes), *edr1* (enhanced disease resistance) and *lsd* (lesion stimulating disease) inactivation does. However other mutations compromise the defense response; the mutants providing SA-signaling deficiency are the *npr* (non-expressor of *PR* genes)/*nim* (non-inducible immunity), *sai* (SA insensitive); *pad4* (phytoalexin deficient),

*eds1* (enhanced disease susceptibility); the mutant with ET-signaling deficiency is the *ein* (ethylene insensitive); with JA-signaling deficiency is the *coi1* (coronatine-insensitive). All of these mutant plants displayed increased susceptibility to pathogen infections, meaning that these signaling pathways are also components of defense reaction (Yang et al., 1997; Brodersen et al., 2002; Wang et al., 2002; Glazebrook, 2005; Katsir et al., 2008). Functional screens on these mutants provide better insight into the role of defense genes in signaling pathways.

### Genes that regulate plant responses

To trigger the immune response the pathogen needs to be recognized by R proteins. The R genes are usually dominant alleles and may be categorized into four groups based on their function: (i) R genes, which are direct targets in the gene-for-gene interaction; (ii) the genes that support the target in this interaction; (iii) the genes that recognize the PAMPs; (iv) the genes that have detoxification function (Figure 2). The R genes regulate robust resistance against a specific pathogen or a pathogen race. However this robustness is maintained as long as a new virulent strain does not try to infect the plant. Beside the R gene mediated resistance the Quantitative Disease Resistance (QDR) loci are encoded by multiple genes (quantitative trait loci/QTL), therefore these are more effective traits to evade pathogens. The selection pressure on pathogen races is lower, therefore the QDR provides more

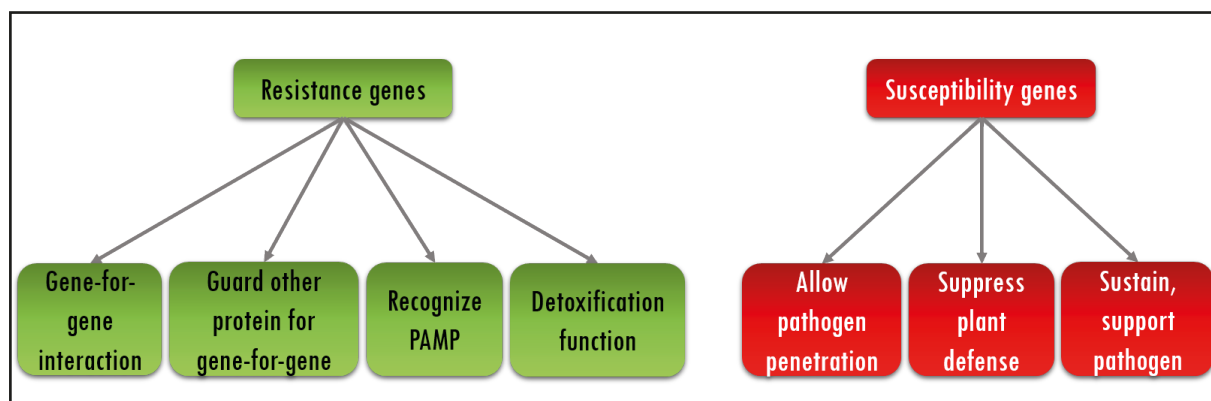
lasting resistance to diseases than R genes (Parlevliet, 2002). Discovery of R genes or QDR-s and then integrating them into susceptible variety is an efficient procedure supported nowadays by MAS.

In contrast to the dominant resistance genes, the susceptibility genes increase resistance if they lose their function; therefore these genes are beneficial to enhance pathogen tolerance only in recessive form. The susceptibility genes may be categorized into three groups based on the timing of pathogen support (i) the genes, which allow accommodation of the attacker; (ii) the genes, which suppress defense response; (iii) and the genes, which aid the pathogen to be supplied by nutrients/ water (Figure 2).

### Plant resistance genes

As the first key element of PTI, the pattern recognition receptors (PRR) detect the PAMP, DAMP or MAMP of pathogens. The first discovered PRR was the *Xa21* gene in *Oryza sativa*, which encodes both extracellular LRR (leucine-rich repeat) and transmembrane protein kinase (Song et al., 1995). This protein complex is responsible for recognition of Ax21 (activator of Xa21 triggered immunity) peptide of bacterium *Xanthomonas oryzae* pv. *oryzae* (Lee et al., 2009). After detection, *Xa21* induces intracellular defense response. In plant breeding integration of a single locus of this gene resulted increased resistance to several bacterial blight isolates (Wang et

Figure 2. Function based categories of resistance and susceptibility genes.



al., 1996; Zhai et al., 2002). As an example for fungal PAMP, the chitin of the pathogen cell wall can be recognized by the CEBiP transmembrane protein and induces immunity in rice (Zipfel and Robatzek, 2010). Similarly to this, the Elongation Factor Tu (EF-Tu) bacterial peptide can be detected by the plant EF-Tu receptor (EFR). *EFR* belongs to the same subfamily as the leucine-rich repeat-receptor-like protein kinase (LRR-RLK), the FLS2 (flagellin-sensitive 2), which was also found to regulate defense responses (Zipfel et al., 2006). Insertion of the *EFR* gene into the wheat genome enhanced resistance to bacterial diseases (Schoonbeek et al., 2015). FLS2 protein was found to be ET-dependent, and to be integrated into the plasma membrane. FLS2 bound to the bacterial flagellin 22-amino-acid epitope (Flg22) at the early stage of bacterial infection. This interaction with Flg22 regulated FLS2 to associate with BAK1, another LRR-receptor-like kinase. The FLS2-BAK1 complex then activated *BIK1* (*Botrytis-induced kinase 1*) gene, which triggered the mitogen activated protein kinase (MAPK) cascade to govern the defense response (Veronese et al., 2006; Nicaise et al., 2009; Lu et al., 2010). *BIK1* was found to positively manage defense against necrotrophs, but repressed the response to the virulent biotrophic bacteria *Pseudomonas syringae* pv *tomato* (Veronese et al., 2006). Additionally, the FLS2 physically associated also to the resistance proteins RPM1, RPS2 and RPS5, which all regulate in ETI (Qi et al., 2011). This fact proves that PTI and ETI signaling components overlap (Thomma et al., 2011).

The *R* genes, which encode proteins for 'gene-for-gene' interaction usually belong the *NBS-LRR* superfamily. These genes code for a central nucleotide binding site (NBS) and leucine-rich repeat (LRR) domain at the C-terminal. These proteins are categorized into two groups based on the N-terminal domain: (i) the TIR (Toll/Interleukin-1 receptor-like domain) group genes and (ii)

the non-TIR or Coiled-coil (CC) NBS-LRRs (Gururania et al., 2012). NBS-LRR proteins play a role in regulation of the effector triggered immunity. They may bind directly to the pathogen effector or guard other protein for completion the interaction in order to induce defense response (DeYoung and Innes, 2006). For example the tomato *Bs4* TIR-NBS-LRR complex detected directly the AvrBs4 effector protein of *Xanthomonas campestris* pv. *vesicatoria*. *Bs4* represents high homology to the tobacco *N* and potato *Y-1* resistance genes (Schornack et al., 2004). The barley *Mla1* and *Mla6* genes were found to be active in powdery mildew (PM) infection in barley. These genes code CC-NBS-LRR proteins; especially the *Mla6* activated *RARI* and *SGT1* resistance genes for induction of immunity against PM (Shen et al., 2003). Furthermore the *RARI* was required for activation of the tobacco *N* gene against Tobacco Mosaic Virus too (Liu et al., 2002). The Pi-ta CC-NBS-LRR protein directly interacted with the rice blast fungus AVR-Pita effector. Only a single amino acid change in the protein altered resistance trait to susceptibility (Bryan et al., 2000). The *RPW8* gene, found in *Arabidopsis*, code an N-terminal transmembrane protein and a coiled coil domain. The *RPW8.1* and *RPW8.2* regulated defense response through the SA-mediated signaling, and associated with *PAD4*, *EDS5*, *NPR1* and *SGT1b* defense genes for activation PM resistance and HR. These *RPW8* genes are independent of *COI1*- and *EIN2*-mediated signaling pathways. However the *edr1* mutation, which repress the SA-signaling, lowered the activity of *RPW8.1* and *RPW8.2* and programmed cell death too (Xiao et al., 2005). The tomato *Cf* genes has been used for decades to improve resistance in crop plants. These genes code extra-cytoplasmic LRRs and C-terminal membrane anchor (Jones et al., 1994). The *Cf-4* gene, following the interaction with Avr4 effector of *Cladosporium fulvum*, triggered ETI and HR in tomato. Interestingly, *Cf-4* also recognized homologous cognate effector

proteins secreted by multiple pathogen species (Stergiopoulos et al., 2010). Most of the *R* genes encoding NBS-LRR proteins are putatively targeted in cytoplasm. However the *Arabidopsis thaliana* *RRS1-R* gene coding a TIR-NBS-LRR complex harbors a nuclear localization signal and a WRKY-type DNA binding domain at the C-terminal extension. Several hypothesis are, how this protein activates defense against the wilt (*Ralstonia solanacearum*): (i) the PopP2 effector contains also a nuclear localization signal, in this way the interaction with *RRS1-R* is achieved inside the nucleus; or (ii) the *RRS1-R* is located in the cytoplasm in inactive form, and following the interaction the complex of *RRS1-R*-PopP2 is transmitted together into the nucleus (Lahaye, 2002; Deslandes et al., 2003). As an example for guarding other protein, the tomato Pto intracellular Ser/Thr protein kinase activated ETI along with guidance of Prf-TIR-NBS-LRR protein. Pto interacted directly with AvrPto or AvrPtoB elicitors secreted by the bacteria *P. syringae* pv. *tomato* (Oh and Martin, 2011). Discovery of *R* genes increases the evidence that resistance proteins rather guard a few host protein for effector recognition, than direct contact with pathogen secreted proteins. Based on the guard model a single *R* protein may be able to interact with multiple effectors and other *R* genes are transcribed in order to guide their interaction and trigger immunity (Zhang et al., 2013).

Host selective toxins (HSTs) are effective weapons of necrotrophs to kill the plants. These phytotoxins generate necrotic lesions in plant tissues and forward colonization of the pathogen. Phytoalexins are then accumulated in order to evade the toxic effect of HSTs. For example the camalexin phytoalexin accumulated in response to *Alternaria brassicicola* infection in *Arabidopsis*. (Saga et al., 2012). Furthermore camalexin also increased resistance against *Botrytis cinerea* and *Leptosphaeria maculans* (Bohman et al., 2004). Another gene family with antimicrobial

activity are the defensins, the small cysteine-rich molecules. These compounds are able to inhibit the virulence of microorganisms directly by alteration of the fungal membrane permeability, or may enhance plant innate immunity by triggering programmed cell death (Aerts et al., 2008; Hegedüs and Marx, 2013). These genes are widespread among the plants, insects and mammals, therefore they probably have common ancestral origin. The plant defensin proteins inhibit colonization of a broad range of filamentous ascomycetes, such as *Fusarium graminearum*, *B. cinerea*, or *A. brassicicola* (de Zelicourt et al., 2007; Stotz et al., 2009; Sagaram et al., 2011). Especially the *PDF1.2* plant defensin gene was found to be JA-dependent; the JA signaling-deficient mutant depressed the *PDF1.2* expression and represented high susceptibility to necrotrophic fungus (Veronese et al., 2004). *PDF1.2* probably depends on *BIK1*-mediated pathway also, because induction of the gene was significantly lower in the *bik1* mutant in response to pathogen infection, than in wild genotype (Veronese et al., 2006). Plant are able to express genes, which directly deactivate hazardous compounds of the pathogen. As an example a specific detoxification gene found in maize, the *Hm1* encoding HC toxin reductase neutralized the cyclic tetrapeptide toxin produced by *Colchiobolus carbonum* (Johal and Briggs, 1992).

#### *Quantitative disease resistance loci*

The quantitative resistance loci (QRLs) provide broad-spectrum and long-lasting resistance against microorganisms. More than hundred resistance loci are found in wheat against *Fusarium* head blight (FHB) already (Buerstmayr et al., 2009). In a breeding experiment 19 QTL-Near Isogenic Line pairs (with *Fusarium* resistance locus) developed by microsatellite markers showed significant resistance to head blight (Pumphrey et al., 2007). In 'Arina' wheat cultivar the resistance QTL to FHB was localized on chromosome 4D, which results in stable resistivity against

the fungus (Draeger et al., 2007), while according to another study using the GK Mini Manó/Fontana DH population the FHB resistance loci of 'Frontana' are located on chromosomes 1B, 2D, 3B, 5A, 5B and 6B (Szabó-Hevér et al., 2014). Recombinant Inbred Lines (RIL) were developed and used to test tolerance to barley leaf rust (*Puccinia hordei*) by crossing the susceptible 'L94' and resistant 'Vada' varieties. The lines contained six QRLs (*Rphq1-Rphq6*), which were found to be responsible for partial resistance against *P. hordei* (Qi et al., 1998). The head smut is a serious disease of maize, the *qHSRI* QRL provides resistance against it without any change in other agronomic traits (Zhao et al., 2012). The *Sr36/Pm6* gene cluster resulted in significant resistance in wheat cultivars against stem rust (Purnhauser et al., 2011). Studies of resistance to powdery mildew in cultivated grapevine demonstrated that single dominant genes (*RENI*, *RUN1*) regulate responses to evade pathogenic infection (Molnár, 2007; Hoffmann et al., 2008; Kozma et al., 2009; Katula-Debreceni et al., 2010; Li et al., 2013). Especially, the *RENI* was found to co-segregate with the NBS-LRR gene cluster in 'Dzhandhzal kara' and 'Kishmish vatkana' resistant *Vitis vinifera* varieties (Coleman et al., 2009). Adaptation of QDRs along with *R* genes may maintain a broad range defense system for susceptible plants.

#### *Plant susceptibility genes*

Pathogens enter the plant cell by punching the cell wall, or intrude through wounds and leaf stomata. These entry processes may also be achieved by the assistance of plant. Many genes are identified to be required in the host for compliance of infection. If that certain gene is not carried by the plant, the pathogen may not be able to infect it. The first discovered *S* gene was identified in barley, called the *Mildew Resistance Locus O*, *MLO* (Jørgensen, 1976). The *MLO* seven transmembrane protein is integrated into the cell membrane and supports development

of haustoria of filamentous biotrophs. This *S* factor requires  $Ca^{2+}$  and calmodulin to suppress defense responses (Ayliffe and Lagudah, 2004), and is independent from JA/ET or SA-mediated signaling (Consonni et al., 2006). The loss of function *mlo* resulted an interaction between syntaxin, SNARE (Ror2) and SNAP (HvSNAP34) proteins and promoted the membrane vesicle fusion (Ayliffe and Lagudah, 2004). This vesicle trafficking increased resistance to *Blumeria graminis* fsp. *hordei* powdery mildew and other pathogens resembling a 'non-host resistance' trait (Humphry et al., 2006). Similarly to *MLO* the BAX inhibitor-1 (BI-1) protein compose a six or seven transmembrane complex, it allows the penetration of *B. graminis*, and additionally it suppresses programmed cell death (Eichmann et al., 2004; Eichmann et al., 2010). Interestingly the overexpression of this gene restored the PM penetration success in *mlo* mutants, as well as *MLO* overexpression in *bi-1* mutants (Huckelhoven et al., 2003). BI-1 protein belongs to the Lifeguard protein family, in which the members were found to negatively regulate the cell death too (Hu et al., 2009). The modulation of the cell surface may also limit the invasion of the attacking organism. The decreased very-long-chain aldehyde levels of *glossy11* maize mutant leaf surface inhibited PM spore germination (Hansjakob et al., 2011). Similarly the *irg1* and *ram2* mutations altered the *Medicago* leaf cuticle layer, therefore it became resistant to several pathogens (Uppalapati et al., 2012; Wang et al., 2012).

Expansins are used by plant for cell wall growth and stretch. The expansin *EXLA2* provide susceptibility to *B. cinerea* and *A. brassicicola*, probably by enabling pathogen entry. Mutation in *EXLA2* resulted in an additional side-effect; it increased hypersensitivity to abiotic stresses (Abuqamar et al., 2013). The cellulose synthase-like (*CSLA9*) gene was required for *Agrobacterium* attachment to the plant root surface, suggesting that *CSLA9* is an essential

cue for host recognition (Zhu et al., 2003). The *AtCLCd* chloride channel encoding gene repressed the Flg22-triggered immunity in *Arabidopsis*. The T-DNA insertion 'knock-out' mutants represented enhanced response to Flg22, and increased resistance to virulent strain of the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (Guo et al., 2014). The small G proteins genes (RAC/ROP) also regulate vesicle trafficking; among them the *HvRAC1*, *HvRAC3*, and *HvROP6* encode susceptibility factors in barley. Overexpression of these genes increased the sensitivity to PM infection to a greater extent (Schultheiss et al., 2002; Pathuri et al., 2008). The orthologs identified in rice (*OsRAC4*, *OsRAC5*, *OsRACB*) acted as compatibility factors also in response to the adapted fungus, *Magnaporthe oryzae* (Jung et al., 2006; Chen et al., 2010a). However the *HvRAC1* provided resistance against the non-adapted *M. oryzae* in barley (Pathuri et al., 2008), which indicates specificity of the gene to the attacker. A thiopurine methyltransferase (ubiquitin-conjugating enzyme), and an ADP ribosylation factor-GTPase-activating protein (ARF-GAP) acted as candidates of *Blumeria graminis* f. sp. *hordei* effector molecule (Schmidt et al., 2014). Schmidt and co-workers suggests that the *ARF-GAP* vesicle trafficking genes are conserved targets of mildew effectors. Taken together, the genes mediating cytoskeleton rearrangements and vesicle trafficking (*MLO*, *LFG*, *BI-1*, *ROP*, *RAC*) are responsible for sensitivity to adapted but resistance to non-adapted fungi (van Schie and Takken, 2014). Pathogens may sustain infection by inhibition of defense signaling or response. The SA signaling obtains key role in defense system against biotrophs, therefore enhancing this pathway increases resistance. The SA 3-hydroxylase enzyme degrades SA by conversion it into 2,3-DHBA in *Arabidopsis*. The mutation in gene encoding this enzyme resulted in SA accumulation and increased tolerance against *P. syringae* (Zhang et al., 2013). SA signaling is escalated in response to biotrophs, but not

to necrotrophs. The bHLH3/13/14/17 (basic loop-helix-loop) transcription factors found to suppress JA signaling; the quadruple knock-out mutant expressively increased innate JA and resistance to *B. cinerea*. However due to the antagonistic relation (Robert-Seilaniantz et al., 2011), in these plants the JA signaling was intensified along with repression of SA pathway, therefore the appearance of susceptibility to biotrophic pathogen (Song et al., 2013). Mutation in cellulose synthase genes activated the JA and ET mediated defense responses, and enhanced resistance against pathogens (Ellis and Turner, 2001; Ellis et al., 2002; Hernandez-Blanco et al., 2007). Interestingly, in this case the decreased cellulose content triggered the immune response. This is probably in association with accumulation of oligogalacturonides (cellulose precursors), which mimic DAMP and trigger DTI (van Schie and Takken, 2014). Similarly to this, although the increased callose content benefit resistance, *pmr4* callose synthesis inhibited mutant also showed decreased susceptibility to PM species. The accumulated oligosaccharides - which are able to induce the DTI - can be the explanation for this (Nishimura et al., 2003). The *PMR4* down-regulation generated a total resistance to the adapted, but not to the non-adapted fungi (Jacobs et al., 2003; Huibers et al., 2013).

Sequentially, after recognition of the pathogen, the phosphorylation-mediated MAP kinase cascade activates the response to biotic stress. Therefore the molecules inactivating the cascade components can be considered as susceptibility factors too. The MAPK phosphatases (MKPs) dephosphorylate the cascade components, in this way abolish its function. The *MKP1* and *MKP2* loss of function mutant provoked decreased susceptibility to virulent *Ralstonia* and *Pseudomonas* bacteria (Bartels et al., 2009; Lumbreras et al., 2010; Anderson et al., 2011). On the contrary, some MAPKs repress PTI; the *MPK4* of soybean and *MAPK5* of rice reduced activity, therefore

the effectiveness of PTI. The mutation in these genes resulted in increased resistance to several pathogens (Xiong and Yang, 2003; Liu et al., 2011). The *enhanced disease resistance 1 (EDR1)* locus encodes a putative MAPKK kinase, which was found to negatively regulate the SA-mediated responses in *Arabidopsis* (Frye et al., 2001). However it also depends on the ethylene signal, since the *ein* mutation altered the expression of *EDR1* in response to senescence. The *EDR1* probably acts in a cross-talk between ET and SA-mediated pathway operating in cell death and ageing (Tang et al., 2005). Followed the activation of MAPKs-mediated cascade, the transcription factors (TFs) actuate the defense reaction. The mostly active TFs during infection are the WRKY transcription factors, which were found to positively or negatively regulate defense. Especially the rice *WRRKY45-2* gene acted as susceptibility factor against *X. oryzae*, but the homolog gene, which differs only in a few amino acids positively regulated defense against the same pathogen (Tao et al., 2009). The *Arabidopsis AtWRKY18/40/60* regulatory genes played a role in tempering the SA-mediated defense pathway. Double or triple mutants increased resistance to biotrophic *P. syringae* and susceptibility to *B. cinerea* compared to wild-type plant (Xu et al., 2006). Another transcription factor, the TaNAC21/22 was found to negatively regulate defense against stripe rust, *Puccinia striiformis* f. sp. *tritici*. This *NAC* gene was the target of taemiR164 microRNA, and this miRNA was found in earlier studies to regulate defense responses (Feng et al., 2014). The calcium-/calmodulin- and lipid-binding proteins also suppress the defense reaction in host plants. The SR1 calmodulin-binding transcription factor repressed the immunity by directly binding to the *EDS1*, *NDR1* and *EIN3* promoters (Du et al., 2009; Nie et al., 2012). The lipids act as signaling molecules and are required for ETI and HR (Andersson et al., 2006). The lesion mimic mutant, *acd11* had limited sphingosine (a sphingolipid) transfer protein content

increasing the cellular SA level and resistance to biotrophs. Similar function was found with the sphingolipid fatty acid hydroxylase gene, the *AtFAH1/2* (Brodersen et al., 2002; Konig et al., 2012). The SA-mediated defense response is suppressed by fatty acid desaturase (*FAD7*). In the *fad7* mutant the basal SA level did not show alteration, but in response to aphid attack, the SA accumulated along with enhanced defense (Avila et al., 2012).

Once the pathogen passes the plant defense barriers, the plant is forced to sustain the attacker. Additionally these organisms are able to manipulate plant metabolism to fulfill their nutritional needs and to facilitate their replication and spread. Maintenance involves the modification of host sugar transport; the cell membrane localized sugar transporters (*SWEET11* and *SWEET13*) were forced by *X. oryzae* to transfer more sugar into the intercellular region. Mutation in these genes abolished *X. oryzae* proliferation (Chen et al., 2010b). *SWEET11* associated with copper transporter, *COPT1*, which was also required for susceptibility to *X. oryzae* (Yuan et al., 2010). The alcohol dehydrogenase gene (*ADH*) was up-regulated by PM in barley, however the mutation in *ADH* inhibited PM proliferation (Pathuri et al., 2011). Lipids may also be utilized by the pathogens; the maize *lox3* lipoxygenase mutant plant became full resistant to three different fungus genus. The *lox3* inactivation also blocked the toxin production of *Fusarium* (Gao et al., 2007). The reason of the increased resistance to biotrophs may be explained by the repressed JA synthesis in mutant plant. Therefore inhibition of lipoxygenase activity depress JA synthesis and SA signaling can be enhanced along with defense against biotrophs (Gao et al., 2009).

Hypertrophy and endoreduplication of plant cell also benefit pathogens maintenance. The increased cell size extends the nutrient or water content; the endoreduplication results in multiplication of chromosomes override

overall metabolism of the host. *Xanthomonas* infection induced cell size enlargement in pepper by triggering bHLH transcription factor Upa20 activity via its AvrBs3 effector (Kay et al., 2007). The genes *PMR5* and *PMR6* encode pectate-lyases, which play a role in completion the accommodation of PM haustorium. The presence of these genes is required at later stage of infection and they are independent of SA-mediated signaling (Vogel et al., 2002; 2004). Additionally recent study represented that these genes were influenced by the pathogen to modulate ploidy level of mesophyll cells under the infected, haustorium containing epidermal cells. Therefore the metabolic capacity could be enhanced at the site of infection (Chandran et al., 2010; 2013). Intriguingly, although in *pmr5* and *pmr6* mutants the penetration efficiency was not repressed, the fungus developed less hyphae, conidiophores, and conidia (Vogel et al., 2002; 2004). Substantial study observed that cell cycle regulatory genes were up-regulated in mesophyll cells at infection sites: some cyclin-dependent kinases (CDKs), CDK inhibitors and the *MYB3R4* transcription factor, known to be regulator of G2/M transition. The mutation in *MYB3R4* and in *PUX2* (plant ubiquitin regulatory X domain-containing protein 2) abolished the endoreduplication along with weakened *Golovinomyces orontii* colonization (Chandran et al., 2010). The plant susceptibility factors may support pathogen replication as the eIF4E cap-binding and eIF4G scaffold protein do (Kawaguchi and Bailey-Serres, 2002). These proteins are part of the host translation initiation complex and are forced by potyviruses to translate viral RNA too. The viral RNA 5' end is covered by the viral VPg protein, which interacts with the plant eIF4E (Wittmann et al., 1997). Additionally eIF4E also acted as susceptibility factor of melon tomosvirus and *Arabidopsis* bromovirus (Yoshii et al., 2004; Nieto et al., 2006). Isoforms and mutations of eIF4E and eIF4G were found in many plant species that

abolish susceptibility to potyviruses (Wang and Krishnaswamy, 2012).

Using single marker association and linkage disequilibrium analysis a powdery mildew susceptibility locus (*Sen1*) was identified in 'Chardonnay' grape (*Vitis vinifera* L.), which can be applied for negative selection in breeding programs (Barba et al., 2014). Since the *S* genes mostly possess primary function, disabling the *S* factor may result in negative side-effects beside the desired decrease in susceptibility. In several cases the mutation increases resistance to biotrophs along with susceptibility to necrotrophs (Veronese et al., 2006; Lai et al., 2008) and vice versa (Flors et al., 2007; Mang et al., 2009). It is likely the result of the antagonistic relation between SA- and JA/ET-mediated signaling and their regulating elements. Along with the enhanced susceptibility to other pathogens, other phenotypic changes may also occur, such as stunted plants, susceptibility to abiotic stresses (Bessire et al., 2007; Tang et al., 2007). Therefore monitoring the pleiotropic effect mediated by inactivation of an *S* gene is highly recommended. If the new trait was tested in other plant species, it may act totally differently in the desired genetic background. Additionally the new variety should also be evaluated in field conditions, because interaction with beneficial microbes or resistance-breaking pathogens may alter the performance (van Schie and Takken, 2014). As an example, the *mlo* and *eIF4E*-based resistance have been used for many decades (Cook, 1961; Jørgensen, 1992; Moury and Verdin, 2012). Although the *mlo* provided penetration block it did not result in any resistance-breaking pathogen strain, the *eIF4E* raised new resistance-breaking potyviruses with mutation in viral protein VPg (Masuta et al., 1999).

### Perspectives, breeding strategies

Overexpression of *R* genes, or T-DNA knockout of *S* genes requires genetic transformation of the



plant, which is usually done by *Agrobacterium* or the gene-gun. The current GMO regulation in the European Union discourages the application of transgenic plants in agriculture. The GMO legislation of EU declares that the evaluation of crops is based on the method used for breeding, instead of agronomic value of the new variety. The regulation allows MAS method for searching and integration of *R* genes or QDRs into susceptible variety. However in some cases the QDRs provide only partial resistance, in turn the *R* genes are race-specific. The monogenic, *S* gene-based defense covers more durable resistance to multiple diseases. Especially the obligate biotrophs depend strongly on function of host factor, such as the biosynthesis of essential metabolites, therefore the inactivation of these compatibility factors disables pathogen

invasion. The recent inventions provide good solutions for inactivation of the specific susceptibility gene without T-DNA insertion. The transcription activator-like (TAL) effector nucleases of pathogens may be customized to introduce mutation at designated location of the *S* gene (Bogdanove, 2014). Ironically the pathogen virulence factor can now be modified to apply against them. Since the breeding technology develops exponentially, the recent GMO principle, as well as the regulation of the applied techniques should be under debate (Hartung and Schiemann, 2014).

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## EFFECT OF PLANT DENSITY AND ROW SPACING ON MAIZE (*ZEAMAYS* L.) GRAIN YIELD IN DIFFERENT CROP YEAR

Eszter MURÁNYI

Institute of Crop Science, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen H-4032 Debrecen Böszörményi u. 138. E-mail: emuranyi@agr.unideb.hu

**Abstract:** Nowadays the aim of crop production is to realize the highest possible yield per production area unit by the highest possible crop safety. One of the possible ways for this is to increase of plant density or to decrease row spacing. Therefore, the plant number per unit area can be increased and the potential yield loss of individual plants is compensated by the higher plant density.

The development of yield amounts of eight different genotypes was investigated in a small-plot field experiment with four replications on a calcareous chernozem soil type at the Látókép Research Site of the University of Debrecen in the crop years 2013 and 2014. Row distances of 45 and 76 cm, just as plant densities of 50 000, 70 000 and 90 000 plants per ha were set.

Significant differences were found between the yield amounts of the studied hybrids in both studied crop years, while the effect of plant density on yield amount showed different results. In the crop year of 2013 the hybrids resulted high yields in the treatment with a row distance of 45 cm and plant density of 90 000 plants per ha, however, in 2014 significant yield decrease was found in comparison with the previous year, that can be attributed to the weather conditions in the months April-May and June.

Optimal plant densities of hybrids, just as the corresponding expectable yield amounts were determined with quadratic equations. Optimal plant densities of the hybrids were different in the two studied crop years: in 2013, regarding the treatments set with the row distance of 45 cm, increasing plant densities resulted in higher yields, while in 2014, the yield showed decreasing tendency parallel to the increasing plant densities, that is confirmed by the fact that plant densities of 50 000 and 65 000 plants ha<sup>-1</sup> proved to be more favourable. Regarding the treatments with a row distance of 76 cm, hybrids obtained their yield maximums by 80 327 plants ha<sup>-1</sup> in 2013, while in the vegetation of 2014, by higher plant density (85 845 plants ha<sup>-1</sup>).

**Keywords:** maize, grain yield, plant density, row spacing

### Introduction

According to Pepó and Sárvári (2013), maize is a plant with individual productivity; therefore plant density determines yield significantly. Optimal plant density can be affected by the genetic properties and vegetation time of the given hybrid, just as by the conditions of the production area, by the crop year and the extent of water and nutrient supply. Sárvári et al. (2002) found that different hybrids endure production using higher plant densities in different extent. Plant density is a production factor that affects yield to the greatest extent. Parallel to the increasing plant density the individual production of plants decreases but the yield per unit area increases, however to a certain limit.

Roekel and Coulter (2011) determined a close relationship between maize yield and plant

density. The studied hybrid produced maximal yield by a plant densities of 81 700 plants ha<sup>-1</sup> or even higher. On the basis of their research work Berzsenyi and Lap (2005) have found that optimal plant density varied between 67 483 and 70 161 plants ha<sup>-1</sup> regarding the average of the involved hybrids.

Nagy (1983) concluded based on his research results that the plant density of 60 000 plants ha<sup>-1</sup>, which was considered to be optimal, could be effectively increased up to 80 000 plants ha<sup>-1</sup>, in case row distance would be reduced from 70 cm to 50 cm. According to Shapiro and Wortmann (2006) a yield increment of 4% could be produced by decreasing the row distance from 76 cm to 51 cm. Widdicombe and Thelen (2002) stated that yield increased by 2-4% as the result of decreasing row spacing from 76 cm to 56 and 38 cm. According to the conclusions of Andrade et al. (2002), Lutz

Table 1. The values of monthly precipitation (mm) and temperature (°C) in maize growing season and in the previous period (Debrecen, 2013. 2014.)

Debrecen: Precipitation and mean air-temperature (61-90: 30-year means 1961-1990)								
Year	Jan.- March.	April	May	June	July	Aug.	Sept.	April-Sept.
Precipitation (mm)								Total
2013	228	48	69	31	16	32	48	243
2014	77	40	69	8	128	45	96	385
<b>61-90</b>	<b>101</b>	<b>42</b>	<b>59</b>	<b>80</b>	<b>66</b>	<b>61</b>	<b>38</b>	<b>345</b>
Mean air-temperature (°C)								Mean
2013	1.4	12.0	16.6	19.6	21.2	21.5	14.0	17.5
2014	4.8	12.3	15.4	19.0	21.2	19.8	16.7	17.4
<b>61-90</b>	<b>0.9</b>	<b>10.7</b>	<b>15.8</b>	<b>18.7</b>	<b>20.3</b>	<b>19.6</b>	<b>15.8</b>	<b>16.8</b>

et al. (1971), the decrease of row spacing resulted in yield increment as well. Fanadzo et al. (2010) found that the application of 45 cm row distance resulted in 11% higher grain yield than in case of the setting of 90 cm rows. Increasing plant density from 40 000 to 60 000 plants ha<sup>-1</sup> resulted in 30% higher grain yield. In the study of Giesbrecht (1969) row spacing (50, 65, 80 and 90 cm) did not affect grain yield. Varieties showed significant differences in their yield response to increased plant densities.

In their research work Hunter et al. (1970) found that the yield of all studied hybrids increased parallel to each increasing plant density setting and showed low but significant increments when narrowing the row spacing. Gozübenli et al. (2004), just as Lashkari et al. (2011) found that plant density has significant effect on yield.

Yield showed increasing tendency up to a plant density of 90 000 plants ha<sup>-1</sup> (10 973 kg ha<sup>-1</sup>), but by any higher density it decreased. Hoshang (2012) also found that there were significant differences between the yields of different plant populations which increased with increasing plant density. In his work Mohseni et al. (2013) confirmed that the increase of plant density from 60 000 plants ha<sup>-1</sup> (9.09 t ha<sup>-1</sup>) to 80 000 plants ha<sup>-1</sup> (11.14 t ha<sup>-1</sup>) resulted in a yield increment as well.

## Materials and methods

The small-plot field experiment was set up with four replications at the Látókép Research Site of the University of Debrecen in the crop years of 2013 and 2014 on a calcareous chernozem experimental soil type which is characterized by good nutrient management due to its favourable nitrogen supply, phosphate mobilization and potassium supply capacity. Water management of the experimental soil is considered to be very good, since all soil layers show excellent water permeability and water storing capacity. Pre-crop was winter wheat in both years; PK fertilization was not performed on the experimental area, while N was applied in an amount of 180 kg ha<sup>-1</sup>.

In this present research work the effect of different row spacing (45 and 76 cm) and plant densities (50 000, 70 000, just as 90 000 plants ha<sup>-1</sup>) on yield amounts was studied. Hybrids of the very early (FAO 240-299), early (FAO 300-399) and medium (FAO 400-499) maturity groups were involved in the experiment. The studied hybrids were: Sarolta (FAO 290), DKC 4025 (FAO 330), NK Lucius (FAO 330), PR 37N01 (FAO 380), DKC 4490 (FAO 380), P 9494 (390), Kenéz (FAO 410) and SY Afinity (FAO 470). Weather conditions of the two studied crop years (Table 1.) were different. The amount of precipitation that fell before the vegetation period in 2013 was 332.7

mm. In contrast in the crop year of 2014 only 167.1 mm precipitation fell between October and April. In the vegetation period of maize the amount of fallen precipitation was 242.9 mm in 2013, while in 2014 it was 385.4 mm. The high amount of precipitation in March (136.3 mm) proved to be determining in 2013 regarding the water supply, which filled the water stock of the soil. In 2014, the amount of precipitation in the vegetation period (396.7 mm) did not differ significantly from the long-term average value (378.6 mm), but its distribution was not appropriate. The greatest water deficit was observed in June (7.9 mm).

For the statistical evaluation of the experimental results, software SPSS 13.0 for Windows and Microsoft Excel 2010 were used. The statistical evaluation, the three-factorial variance analysis and correlation analysis were calculated according to the methods described by Sváb (1981) and using regression equations. During the correlation analysis following types of correlations were determined corresponding to the  $r$  values:  $r < 0.4$ : loose,  $0.4-0.7$ : medium,  $0.7-0.9$ : tight,  $> 0.9$ : strong.

### Results and discussion

In the crop years of 2013 and 2014 the effect of the application of different plant densities and row spacing on grain yield amount was studied in case of 8 hybrids of different genotypes and variant vegetation period lengths.

In the crop year of 2013 yield amounts varied between 12.7 and 14.6 t ha<sup>-1</sup> in case of a row spacing of 45 cm, while in case of that of 76 cm between 13.0 and 13.9 t ha<sup>-1</sup>. The highest yields were measured in case of a plant density of 90 000 plants ha<sup>-1</sup> in the average of the hybrids in case of both row distances (Table 2.). The studied hybrids obtained their yield maximums by plant densities of 70 000 and 90 000 plants ha<sup>-1</sup>. There were significant differences between the yields of the involved hybrids.

The difference between the yield amounts corresponding to the two different row

distances was not significant (200 kg ha<sup>-1</sup>). The increase of plant density resulted in significant differences in the yield in the case of both row spacings. The yield increased parallel to the increasing density of the plant population.

The increase of the plant density resulted in relatively higher yield increment by the application of narrow row spacing (45 cm) compared to the common row spacing (76 cm). In the case of the row spacing of 45 cm, increasing the basic plant density (50 000 plants ha<sup>-1</sup>) to 70 000 plants ha<sup>-1</sup> resulted in the highest yield increment: 9.09% (1 283 kg ha<sup>-1</sup>). However, increasing the plant density by further 20 000 plants ha<sup>-1</sup> (90 000) did not result in any expressed yield increment: 3.80% (615 kg ha<sup>-1</sup>). The highest yields were measured in case of the hybrid P 9494 regarding all plant densities. Evaluating the studied hybrids it can be stated that the hybrids DKC 4025 and NK Lucius reached their yield maximums by a plant density of 70 000 plants ha<sup>-1</sup>, while all the others by 90 000 plants ha<sup>-1</sup>.

In the case of the row spacing of 76 cm, the increase of plant density from 50 000 to 70 000 plants ha<sup>-1</sup> resulted in a yield increment of 4.12% (581 kg ha<sup>-1</sup>), while the extent of this increment was 2.46% (353 kg ha<sup>-1</sup>) when plant density was increased from 70 000 to 90 000 plants ha<sup>-1</sup>. In case of the plant density of 70 000 plants ha<sup>-1</sup> highest yield amounts were measured for the hybrids Sarolta and P 9494, while the other hybrids produced maximal yield by plant density of 90 000 plants ha<sup>-1</sup>.

As a summary it can be concluded that the highest yield increment was produced by increasing plant density from 50 000 to 70 000 plants ha<sup>-1</sup> in the case of both applied row spacings. Regarding the average of the hybrids, they showed yield decrement when row spacing was decreased (76 to 45 cm) by 50 000 plants ha<sup>-1</sup>: -2.54% (-323 kg ha<sup>-1</sup>), however, in case of plant densities of 70 and 90 000 plant ha<sup>-1</sup> yield increment was observed parallel to

the decrease of row spacing: 2.72% (380 kg ha<sup>-1</sup>) and 4.4% (642 kg ha<sup>-1</sup>), respectively.

Hybrids of different genotypes showed different responses to the changes in the plant density and row spacing. Most of the hybrids showed to produce a yield increment parallel to the increase of the plant density, while in case of the row spacing of 45 cm, hybrids DKC 4025 and NK Lucius, while in case of that of 76 cm hybrids Sarolta and P 9494 responded to the increase of the plant density by 20 000 plants ha<sup>-1</sup> by yield decrement.

Regarding the average of the involved hybrids yield amounts varied between 10.6 and 11.6 t ha<sup>-1</sup> in case of the row spacing of 45 cm, while in case of that of 76 cm between 11.6 and 14.1 t ha<sup>-1</sup> in the average of the hybrids in the crop year of 2014 (Table 2.). The difference

between the yields corresponding to the two different row spacings was 14.6% (1800 kg ha<sup>-1</sup>). In 2014 the application of different row spacings, plant densities and hybrids resulted in significant differences in the yield amounts. In case of the row spacing of 76 cm, significant difference was measured between the yields by plant densities of 50 000 and 70 000 plants ha<sup>-1</sup>, and similarly in case of both studied row spacings between the yields of the treatments with plant densities of 50 000 and 90 000 plants ha<sup>-1</sup>. The studied hybrids produced maximum yields by plant densities of 50 000 and 70 000 plants ha<sup>-1</sup> in the case of the row spacing of 45 cm; while by that of 76 cm, by plant densities of 70 000 and 90 000 plants ha<sup>-1</sup>. There were significant differences between the yields of the involved hybrids in this crop year as well. The hybrids Sarolta,

Table 2. Impact of row spacing, plant density and hybrid on grain yield of maize

Látókép Exp. Farm: Impact of row spacing (the factor A: 45 and 76 cm), plant density (the factor B: PD in thousand plants ha <sup>-1</sup> ) and genotype (the factor C: hybrids H1-H8) on grain yield of maize																								
H*	The 2013 growing season								The 2014 growing season															
	Row spacing 45 cm				Row spacing 76 cm				Row spacing 45 cm				Row spacing 76 cm											
	PD				PD				PD				PD											
	50	70	90	x	50	70	90	x	50	70	90	x	50	70	90	x								
	Grain yield of maize (t ha <sup>-1</sup> )								Grain yield of maize (t ha <sup>-1</sup> )															
<b>H1</b>	10.8	12.6	12.9	<b>12.1</b>	11.9	12.0	11.8	<b>11.9</b>	10.3	10.1	9.6	<b>10.0</b>	9.6	11.0	12.1	<b>10.9</b>								
<b>H2</b>	11.9	12.3	11.6	<b>12.0</b>	11.9	12.6	13.7	<b>12.8</b>	11.8	11.5	10.4	<b>11.2</b>	11.0	13.1	13.9	<b>12.7</b>								
<b>H3</b>	12.1	14.0	13.7	<b>13.3</b>	13.4	12.9	13.8	<b>13.4</b>	10.8	11.2	10.4	<b>10.8</b>	11.1	13.3	14.0	<b>12.8</b>								
<b>H4</b>	13.3	16.1	17.1	<b>15.5</b>	13.8	15.4	16.3	<b>15.2</b>	11.9	13.1	13.2	<b>12.7</b>	12.6	13.8	16.3	<b>14.2</b>								
<b>H5</b>	11.7	12.2	12.9	<b>12.3</b>	11.9	12.8	13.2	<b>12.6</b>	11.3	10.7	9.8	<b>10.6</b>	11.3	12.7	13.8	<b>12.6</b>								
<b>H6</b>	16.7	17.5	17.7	<b>17.3</b>	15.1	15.6	14.3	<b>15.0</b>	12.4	12.5	12.1	<b>12.3</b>	14.3	15.0	14.0	<b>14.4</b>								
<b>H7</b>	11.3	12.4	13.9	<b>12.5</b>	11.4	11.9	12.4	<b>11.9</b>	9.8	10.1	10.2	<b>10.0</b>	10.2	11.3	13.1	<b>11.5</b>								
<b>H8</b>	13.6	14.6	16.9	<b>15.0</b>	14.7	15.4	15.9	<b>15.3</b>	12.5	13.3	9.3	<b>11.7</b>	13.2	15.1	15.3	<b>14.5</b>								
<b>x</b>	<b>12.7</b>	<b>14.0</b>	<b>14.6</b>	<b>13.7</b>	<b>13.0</b>	<b>13.6</b>	<b>13.9</b>	<b>13.5</b>	<b>11.3</b>	<b>11.6</b>	<b>10.6</b>	<b>11.2</b>	<b>11.6</b>	<b>13.2</b>	<b>14.1</b>	<b>13.0</b>								
	LSD (5% probability level)								LSD (5% probability level)															
	<b>A</b>	<b>B</b>	<b>C</b>	<b>AB</b>	<b>ABC</b>				<b>A</b>	<b>B</b>	<b>C</b>	<b>AB</b>	<b>ABC</b>											
	0.50	0.35	0.51	0.49	1.25				0.85	0.46	0.44	0.65	1.09											
	Correlation coefficients																							
	Grain yield : row spacing								2013 = -0.058;								2014 = 0.448 (**)							
	Grain yield : plant density								2013 = -0.287 (**);								2014 = 0.184 (*)							
	Significance at 0.05 (*) and 0.01 (**) levels																							
	*H1: Sarolta; H2: DKC 4025; H3: NK Lucius; H4: PR 37N01; H5: DKC 4490; H6: P 9494; H7: Kenéz; H8: SY Afinity																							

DKC 4025 and DKC 4490 responded to the increase of the plant density from 50 000 to 70 000 plants ha<sup>-1</sup> with yield decrement; while every studied hybrid responded to the increase of the plant density from 70 000 to 90 000 plants ha<sup>-1</sup> with yield decrease.

In the crop year of 2014 in case of the row spacing of 45 cm yield amount showed decreasing tendency due to the increase of the plant density; while in case of the row spacing of 76 cm the studied hybrids produced the highest yield amounts by the plant density of 90 000 plants ha<sup>-1</sup>, similarly to the previous crop year.

In case of row spacing of 45 cm the population with a plant density of 70 000 plants ha<sup>-1</sup> produced higher yield amount 1.71% (229 kg ha<sup>-1</sup>) compared to that of 50 000 plants ha<sup>-1</sup>. However, yield decrement (-9.62%, -932 kg ha<sup>-1</sup>) was observed when plant density was increased from 70 000 to 90 000 plants ha<sup>-1</sup>. The most balanced yield was produced by the hybrid P 9494. The genotypes Sarolta, DKC 4025 and DKC 4490 showed yield decrement response to the increase of the plant density.

Regarding the row spacing of 76 cm all hybrids but one reached their yield maximums by the plant density of 90 000 plants ha<sup>-1</sup>. High yield increment was resulted by the increase of plant density: 6.51-11.56% (917-1508 kg ha<sup>-1</sup>).

Regarding the average of the hybrids increasing yield decrease tendency was observed as the response to the decrease of the row spacing (from 76 to 45 cm) and the increase of plant density ((-2.76)-(-32.39%), (-313) - (-3 442) kg ha<sup>-1</sup>).

Comparing the two studied crop years, it can be concluded that while hybrids produced high yield amounts by the row spacing of 45 cm even by the plant density of 90 000 plants ha<sup>-1</sup> in 2013, significant yield decrease was observed in 2014 compared to the previous crop year due to the weather conditions in April-May and June. As a result of the different weather conditions in the two studied crop years the yield amount increased by the application of a narrow row spacing (45 cm) of higher plant

density in 2013; while in 2014 the studied hybrids responded with yield decrement to the inappropriate distribution of precipitation.

Difference in the yield amounts between the crop years 2013 and 2014 in an extent of 2.6 t ha<sup>-1</sup> was measured by a row spacing of 45 cm, while this extent was 0.6 t ha<sup>-1</sup> by that of 76 cm. The application of narrow row spacing, the unfavourable distribution of precipitation that did not match the demand of the studied hybrids and the increase of the plant density can be evaluated as stress factors in 2014. In contrast, the application of increasing plant densities by the common row distance resulted in yield increment in both studied crop years.

Beside the increase of the plant density, the optimal plant density that is the most favourable for the applied hybrid under the given conditions shall be determined as well. The optimal plant density was determined corresponding to the maximal yield amounts with regression equations in the range between 50 000 and 90 000 plants ha<sup>-1</sup> (Table 3.).

Optimal plant density of the hybrids and the corresponding expected yield can be determined with quadratic equations. Optimal plant densities of the hybrids were different in the two studied crop years: in case of the row spacing of 45 cm the increase of plant density resulted in higher yields in 2013, while in 2014 yield amount decreased parallel to the increasing plant density, thus plant densities of 50 000 and 65 000 plants ha<sup>-1</sup> were considered as more favourable. In case of the row spacing of 76 cm hybrids produced maximal yields by a density of 80 327 plants ha<sup>-1</sup> in 2013, while in the crop year of 2014 by higher plant density (85 845 plants ha<sup>-1</sup>).

Hybrids that can be produced in a wide or a narrow plant density range can be classified. According to the results of the present research work it can be stated that the hybrids PR 37N01, P 9494 and SY Afinity could be produced in wide, while Kenéz and Sarolta

Table 3. Optimal densities and maximal yields of maize grain of eight hybrids grown in two different row spacing during two growing seasons

Látókép Exp. Farm: Optimal plant density (OPD: plants number ha <sup>-1</sup> ) and maximal yields (t ha <sup>-1</sup> ) of maize grain									
Maize hybrid									
	OPD	Yield	OPD	Yield	OPD	Yield	OPD	Yield	
H1	Sarolta	82,225	12.8	61,875	11.8	51,833	10.3	89,500	12.4
H2	DKC 4025	67,214	12.3	90,000	13.9	50,727	11.7	89,353	13.6
H3	NK Lucius	76,712	13.9	65,875	13.0	66,500	11.2	86,575	13.7
H4	PR 37N01	90,000	17.0	90,000	16.1	84,308	13.5	90,000	15.9
H5	DKC 4490	90,000	12.6	90,000	13.5	50,500	11.2	89,500	14.0
H6	P 9494	84,938	17.6	64,870	15.6	64,250	12.5	67,833	14.9
H7	Kenéz	90,000	14.2	90,000	12.4	87,000	10.3	90,000	13.0
H8	SY Afinity	90,000	16.5	90,000	16.3	63,653	13.7	84,000	15.5
Average		<b>83,886</b>	<b>14.6</b>	<b>80,327</b>	<b>14.1</b>	<b>64,846</b>	<b>11.8</b>	<b>85,845</b>	<b>14.1</b>

in a rather narrow plant density range in case of both studied years and both row spacings. Negative correlation was found between the yield amount and the row spacing in 2013, while in 2014, medium positive correlation proved to be determining (Table 2.). Loose correlation was found between yield and the plant density in both studied crop years.

## Conclusions

Significant differences were found between the yield amounts of the hybrids in the studied crop years, while plant density showed different effects on yield amounts. In the crop year of 2013 hybrids produced high yield amounts even by the row spacing of 45 cm and by plant density of 90 000 plants ha<sup>-1</sup>, however significant yield decrease was observed in 2014 compared to the previous year and the application of lower plant density proved to be more favourable.

Optimal plant density of the hybrids was different in the studied two crop years: in case of the row spacing of 45 cm, increasing the plant density resulted in higher yields in 2013, while in 2014 the yield amounts showed decreasing tendency due to the increase of plant density; thus plant densities of 50 000 and 65 000 plants ha<sup>-1</sup> were considered as

more favourable. In case of the row spacing of 76 cm hybrids produced maximal yield amounts by a density of 80 327 plants ha<sup>-1</sup> in 2013, while in the crop year of 2014 by higher plant density (85 845 plants ha<sup>-1</sup>).

There was negative correlation (-0.058) between the yield amount and the row distance in 2013, while in 2014, medium positive correlation (0.448 (\*\*)) was the determinant. Loose correlation (0.184 (\*), 0.287 (\*\*)) was observed between the yield and the plant number in both years.

The yield determined by quadratic equations regarding the average of the hybrids in case of the row spacing of 45 cm was 14.6 t ha<sup>-1</sup> in 2013, while in case of that of 76 cm it was 14.1 t ha<sup>-1</sup> as well, while in 2014 in case of the row spacing of 45 cm yield amount of 11.8 t ha<sup>-1</sup> was calculated. Considering the present results, it can be concluded that high yield safety can be achieved in case of the application of high plant densities (90 000 plants ha<sup>-1</sup>) by the application of common row distance in crop years with different precipitation supply and distribution. If the distribution of precipitation of the given year is extreme, outstanding yield results cannot be produced even by the application of more closed plant population or in case of a soil type with excellent properties.



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## UTILIZATION OF WASTE FRYING OILS FOR TRANSPORT FUEL EXTENDER – BENEFICIAL TO THE ENVIRONMENT

Valéria NAGY

University of Szeged, Faculty of Engineering, 6725 Szeged, Moszkvai körút 9.;  
E-mail valinagy78@mk.u-szeged.hu

**Abstract:** University of Szeged, Faculty of Engineering carried out research on behalf of Tisza Volán Company (integrated in Centre for South Great Plain Transport Plc.) which takes great care of environmental protection and sustainable management. So the fundamental objective of this research is to analyse utilization possibilities of used vegetable oils (waste frying oils derived from food production, food preparation) as fuels mixed into diesel fuel. This paper summarized the application possibilities, limitations, environmental impacts and difficulties of waste frying oils as fuels. The research points out that all action of different sizes of companies and behaviour of member of society are welcomed and they contribute to sustainable use of environment. This paper helps to expand the knowledge about the utilization of waste frying oils as fuels.

**Keywords:** waste frying oils, fuel, transport

### Introduction

One of the key objective of the European Union developments is energetics, particularly regarding the transport sector: in the energy supply of transport the usage of fossil fuels must be reduced. The ‘Resilient Energy Union with a forward-looking climate change’ means making energy more secure, affordable and sustainable for citizens and businesses. So the possibility of alternative solutions is coming into view such as the more efficient production and utilization of renewable energy. The energy security, environmental and economic challenges can be managed together by the increase of energy efficiency. For public utilization of the achieved results the foundation of European Energy Union also takes place among the energy policy approaches. These intentions are confirmed and served by the fact that among the Social Challenges priority topics of Horizon 2020 Framework Programme appears the establishment of an intelligent, environmentally friendly and integrated transport. This way an energy-efficient and modern transport reduces the dependence on fossil fuels for transport. Furthermore the protection of the natural resources can be facilitated (Horizon 2020). But important principle is that the implement of ‘immature’ technologies does not serve the

fulfilment of objectives but it might induce the opposite effects.

### 1. The necessity of utilization and the criteria of applicability

According to the EU regulations it is also a Hungarian objective to increase the rate of bio-components in both diesel and petrol fuels (42/2005 (10 March); 138/2009 (30 June)).

The current biofuel-rate is 4.8V% that is induced by increasingly stringent environmental regulations, continuous fluctuations of crude oil price, long-term energy policy intentions and last but not least different social and agricultural expectations (Act CXVII of 2010), because the EU has accepted some directives (HTTP1) to facilitate the spread of biofuels. Some of them are important directives about the promotion of the use of renewable sources (2009/28/EC) and also a directive (2009/30/EC) regards about the introducing mechanism to monitor and reduce greenhouse gas emissions particularly regarding the transport services and furthermore, a directive on the promotion of clean and energy-efficient road transport vehicles (2009/33/EC). These directives establish a common framework for the production and promotion of energy from renewable sources and organic wastes and other sources. The CARS 21 work-group has determined in its annual report (in 2012) that

the harmonized alternative fuel-infrastructure is insufficient in EU and it impedes the introduction of alternative fuelled vehicles to the market and delays their favourable environmental effects (CARS 2020). This way an EU regulation was formed with the recommendations of the work-group about the establishment of Connecting Europe Facility (No 1316/2013) and also a directive on the deployment and building of the necessary infrastructure (2014/94/EU).

From the end of the 70's (but the first papers were published in '86/87) intensive experiments were started in the USA and in many European countries (such as Hungary) aiming for the operation of diesel engines with vegetable oil and during them it was confirmed that vegetable oils are suitable for the operation of heavy marine engines and also for lubricating oil (Emöd et al. 1995; MAN 2004; Balog 2005; Carranca 2005; Losoncz et al. 2006; Demirbas et al. 2007; Enweremadu et al. 2009, 2010; Parekh et al. 2012; Naima et al. 2013). Vegetable oils can only be used as diesel engine fuels if they are purified and resin-free. After it such a question was posed whether vegetable oil – that appear in food production – as waste can be utilized for energy purposes. Nowadays frying in vegetable oil is widespread both in the industry and in the households. The Act CXXVII of 2003 contains regulations about the special rules of how to distribute excised products and levy excise tax on petroleum products with waste frying oils used as fuels. According to the Section 50 (2) the petroleum means those products that are produced, offered, sold or used as fuels or additives for fuels or diluent. So according to the Point f) of Section 52 (2) the tax rate can be determined by the tax rate of the closest

petroleum regarding its production, supply, import, offer, sale, or usage as a fuel. Keeping in mind this regulation, *during the research answers were sought for the opportunities of waste frying oils (used vegetable oils) utilised as fuels and in this respect the physical and chemical properties of some waste frying oils were measured and after it bench tests (engine brake tests) and emission tests were carried out – applied different mixing ratios for fuels – on stable diesel engines and on a city bus.*

## 2. The physical and chemical examination of waste frying oils

Thermal and oxidative processes are occurring in the oil during frying at high temperature complex, so the physical and chemical properties of the oil are changing, its colour is becoming darker, its viscosity is getting higher and it tends to foam. In the close past there were some research (Demirbas et al. 1997; Gertz 2000; Aladedunye et al. 2009; Sanli et al. 2011) in order to observe and describe these changes: as a result of polymerization the viscosity increases because of the greater mass of the extended molecules and also acid number, density increases, however, iodine content reduces, peroxide number increases to the limit and then reduces.

The main physical and chemical parameters of the refined sunflower oil and the commercially available diesel fuel according to the standards and product sheet (MSZ EN 590; HTTP2) that are in Table 1. It can be observed that in the case of some parameter (viscosity, flash point) there are significant differences that have impact on the applicability as a fuel.

2 types of frying oils were examined, the samples (control, before and after filtration) can be seen in Figure 1.

Table 1. Important physical and chemical parameters

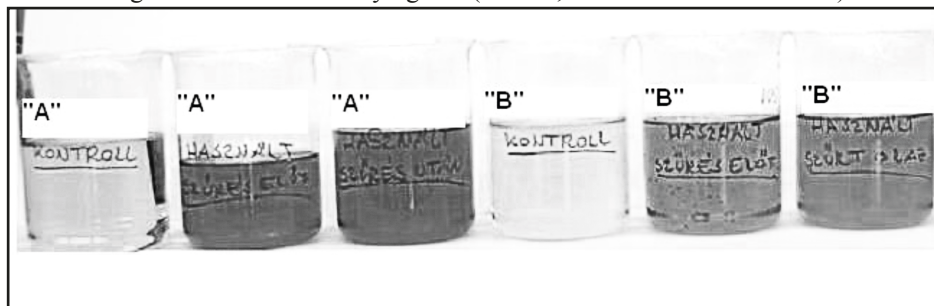
Substance	Density [kg/litre]	Heating value [MJ/kg]	Viscosity at 40 °C [mm <sup>2</sup> /s]	Cetane number [-]	Flash point (open cup) [°C]
Diesel fuel	0.81-0.85	40.6-44.4	2-4.5	>51	>55
Sunflower oil	0.93	37.1	32.9	38	293

The samples main features:

- *Sample "A"*: sunflower oil (was used for one day to fry meat in breadcrumbs)
- *Sample "B"*: sunflower oil (was used for one day to fry meat in breadcrumbs, vegetable and potato)

made on diesel engine fuels. These important general requirements are low volatility, easy pumpability, appropriate viscosity, high resistance to temperature, chemical resistance, easy ignition, proper combustion parameters (cetane number > 50), good flow properties at

Figure 1: "A" and "B" frying oils (control, before and after filtration)



The parameters of the control and used frying oils were measured based on the relevant standards in laboratories at University of Szeged, Faculty of Engineering. The collected waste frying oil samples were first filtered to remove food residues (using 15 µm diameter filter). The filter time (it depends on the composition and viscosity) of 500 ml oil in the case of sample "A": 24 sec/500 ml of sample "B": 22 sec/500 ml.

During the examination of special physical and chemical properties of samples (waste vegetable oils) the main features (Table 2) of each sample were determined – according to the relevant standards – such as density, dynamic viscosity (AND VS vibration viscometer), kinematic viscosity (20 °C), acid number, moisture content, peroxide number. The results were evaluated and compared according to the quality standards and claims

low temperatures, and to be available in a relatively constant quality. *It is expected of fuels to contain components to provide good quality, to be environmental friendly and to have high energy in order to protect the engine and environment.*

The properties of normal diesel fuel were considered during the evaluation of results. So this way it has been determined that the examined filtrated frying oils do not have any significant negative impacts – that would make more difficult their application as a fuel mixed into the normal diesel fuel – compared to diesel fuel.

The filtrated waste oil sample regarding the determined physical/chemical properties there were no significant differences compared to each other. One of the most important parameters is the higher viscosity of vegetable oils. The increase of viscosity is not dangerous

Table 2. The physical and chemical properties of the samples

Samples	Moisture content	Dyn. visc. [Pas]	Density [kg/m <sup>3</sup> ]	Kin. visc. [mm <sup>2</sup> /s]	Peroxide number
"A" control	0.012	0.05473	919.6	59.52	13.53
"A" waste, unfiltered	0.025	0.07835	924.6	84.74	48.26
"A" waste, filtered	-	0.08976	925.3	97.01	45.31
"B" control	0,002	0.05947	919.6	64.67	26.84
"B" waste, unfiltered	0.012	0.06876	922.3	74.55	45.85
"B" waste, filtered	-	0.07723	922.4	83.73	25.83

if the frying oil is dense and viscosity improver processes (polymerization, resinification) are not caused by it. Furthermore, normal diesel fuel reduces the great molecular weight of vegetable oils that is relevant to start combustion and to prevent subsidence in the engine. It must be mentioned here that it is needed to make an analysis (basic combustion parameters) about all waste frying oil before utilization. However, considering the fact that the properties of filtrated frying oil differ from the properties of normal diesel fuel, their combustion properties differ as well and therefore their utilization is limited concerning the conventional diesel engines that were not really designed to be operated with vegetable oil.

### 3. The properties of fuels mixed with waste frying oils

According to the research objective the utilization of waste frying oils as a fuel of internal combustion engines is made by mixing them with normal diesel fuel and therefore the determination of fuel properties produced by different mixing ratios is needed (Table 3). The mixing was carried out by 1/2 part of "A" frying oil and 1/2 part of "B" frying oil. The experimental mixing ratios were the following regarding filtrated frying oils and normal diesel fuels: 0:100; 1:99; 2:98; 5:95; 10:90; 20:80; 100:0.

oil mixed with normal diesel fuel up to a ratio of 10% is applicable as a fuel for vehicles with diesel engines. It can also be mentioned that the examined fuel mixtures do not have any significant negative effects compared to normal diesel fuel and therefore they can be mixed with normal diesel fuels in conventional diesel engines without any problem. The engine brake test (bench test) and operational test justify the hypothesis.

### 4. Results of the engine brake test

As the composition and quality of fuels play a significant role in the operation of heat engines beside the construction design, so it is need to carry out engine brake tests. The tests take place at the University of Szeged where the stable experimental diesel engine is available and their main parts are: conventional VW 1.9 D diesel engine (4-cylinder, cylinder capacity 1896 cm<sup>3</sup>, max power 47 kW, compression ratio 22:1, swirl-chamber engine, indirect injection), control, indicating and data collection system, emission measurement system.

The objective of the tests was to determine the effect of normal diesel fuels with a content of 5% and 10% frying oil on the operation of diesel engines compared to the normal diesel fuelled.

Table 3. Density, kinematic viscosity of experimental samples (at 20 °C)

Mixing ratio [%]	Density [kg/m <sup>3</sup> ]	Kinematic viscosity [mm <sup>2</sup> /s]
0	835.3	3.663
1	837.7	3.736
2	836.0	3.816
5	839.4	4.217
10	843.6	4.943
20	851.4	6.225
100	921.9	69.855

Viscosity increases exponentially (very close correlation) with the mixing of used oil. As viscosity stays under the specified value of 5 mm<sup>2</sup>/s until the used oil content of 10% it can be determined that the filtrated waste frying

Brake tests were carried out according to the ECE R49 standard and the parameters of the engine were determined in relation to engine speed (revolution per minute). The tests contain the full potential operating range. The

engine parameters were determined at different measuring point (ranging from 850-2000 rpm) and then sketched the engine performance curves (power, torque, consumption) (Nagy 2015). Measured properties (exhaust gas temperature, absolute and specific fuel consumption, performance and torque values) were either slightly better or the same as the control samples. Based on the exhaust gas temperatures in relation to engine speed it can be determined that the mixed filtrated waste oils do not influence the temperature conditions of engines. The temperature of exhaust gas was in the range of 250-650 °C in all cases (it should be mentioned that there were lower values at the beginning of the measure). In the range of normal operating engine speed (1000-1800 rpm) the temperature range shrinks and the typical temperatures are 600-650 °C. Operating with normal diesel fuel with a ratio of 5% and 10% waste oil in the range of 850-2000 rpm can interpret almost the same power as in the case of normal diesel fuel. In the case of all the fuels at the engine speed of 1850 rpm the maximum effective power value can be measured.

During the evaluation of torque data it was concluded that the waste frying oil slightly increases the engine torque but the change is not significant. However, this also means that there is not a negative effect on engine dynamics.

Furthermore it can be seen that in the engine speed range of 1400-1800 rpm the specific consumption is nearly constant (260 g/kWh) in the case of all the fuels.

Based on the discussed results the utilization of normal diesel fuels with a maximum ratio of 10% waste oil in internal combustion engines – despite the higher viscosity values – is not difficult and does not cause engine operating problems. According to the previous ones it can be determined that the waste frying oil with a maximum mixing ratio of 10% will change significantly neither the fuel consumption nor

the dynamic properties of the engine so it can be applied under operating conditions. Here it is noted that during operation the engine oil tests need to be performed in order to know the engine oil properties.

### **5. Emission studies on a stable diesel engine**

Based on the physical and chemical properties of mixed engine fuels and also the results of engine brake tests the right quality fuels can be selected in the aspect of engine operation and then fuel tests can be carried out under operating conditions. The tests were carried out with a mixing ratio of 10% waste oil and the goal was to find out what kind of impact has the applied fuel on emission with special emphasis on exhaust fumes. The environmental protection is an important factor in terms of the applicability of fuels with different composition in course of internal combustion engine.

Engine start problems did not occur and the engine ran smoothly during the whole test. Properties of normal diesel fuel were not modified by the mixing of 10%, also smoke and soot were influenced favourably. The operation with mixed fuel in ‘cold’ conditions causes a 50% reduction and in ‘warm’ conditions a 30% reduction regarding smoke compared to the normal diesel fuel operation. A similar trend can be observed during the evaluation of soot where reduction is 50% at the speed of 870 rpm and 30% at the speed of 4000 rpm. These benefits occurred because there is more oxygen in the mixed fuel and the presence of fuel oxygen allows the fuel to burn ‘more completely’, so it can be realized fewer unburned fuel emissions. This way it can be verified that an alternative fuel can be produced from waste frying oil that meets the environmental requirements.

### **6. Trial operation on a city bus, particularly regarding the environmental aspects**

Tisza Volán Company runs about 400 city and interurban buses and coaches to its scheduled

Table 4. Fumigation

	Engine speed [rpm]	Fume [%]	K [m <sup>-1</sup> ]
Measurement I*	2110	29.5	0.81
Measurement II**	2050	23.2	0.61

Note: \*100% normal diesel fuel; \*\*mixture of 92% diesel fuel and 8% waste frying oil

public transport activity. The purchase of significant amount of fuel consumed by vehicles is quite important economic and environmental factor for the company. Therefore the reduction of consumed fuel is a priority task for the company.

University of Szeged and Tisza Volán Company made a research contract to test how the utilization of a mixture of waste frying oil and commercially available diesel fuel oil influences the operation of an internal combustion engine (Final Report 2011).

The selected bus engine specifications: IKARUS 280.40A (year 1998), RÁBA D10UTSLL190, Euro-II environmental class diesel engine, 10350 cm<sup>3</sup>, cylinder capacity, in-line 6 cylinders, turbo-charged, air-recooling, maximum power 190 kW (at 1900 rpm), maximum torque 1130 Nm (at 930-1300 rpm). Used equipments: HFP-100 vehicle testing test bench, BOSCH BEA 85 exhaust gas analyser, AVL DICOM 4000 fumigation measurer.

The trial operation took place at Tisza Volán Company. The nominal capacity of the fuel tank of the bus is 250 litres this way the mixed fuel contained 20 litres of frying oil and 230 litres of normal diesel fuel so the mixing ratio was 8%. The measured values are engine force, power, average fuel consumption and diesel engine exhaust emissions (commonly known as 'diesel fumes'. The soot content (or carbon particle) values depend on the fuel used and the type of engine. Most of the contaminants are adsorbed onto the soot. (So the petrol engines produce more carbon monoxide but much less soot than diesel engines.) Same engine performance, better consumption and substantially better fumigation values

were registered during the measurement of mixing normal diesel fuel with waste frying oil compared to diesel fuelled. The effects of applied fuels on fumigation can be seen in Table 4.

The utilization of mixed filtrated frying oils in road traffic does not influence noticeably the startup and the driving dynamics of the bus, the visible fumigation of the bus is moderate.

### Summary

In the recent years the relations and interactions among environment, economy and society have come to the fore. The applicability tests of alternative fuels such as the recycling of used frying oil can open new possibilities for transport and environmental protection. And it means new challenges for the engineers. But there is an important aspect that the utilization of waste frying oil is only competitive with the conventional fuels if it is considered with its complex advantages. Also the competitiveness of application is assisted by the fact that the mixing does not require engine construction modifications therefore a new option is provided for transport. The advantages of application are the favourable environmental impact and the reduction of energy-dependency. The results of engine tests verify that the filtrated waste oils can be combusted in conventional diesel engines if they are mixed with normal diesel fuel.

It can be the objective of the further research to determine special combustion parameters of other waste frying oils, to observe buses operation and their driving dynamics in public transport under different weather circumstances, and to analyze the impact of mixed fuels on the engine oil parameters.



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138/2009. (VI. 30.) Korm. rend. a bioüzemanyagok közlekedési célú felhasználásának előmozdítására vonatkozó egyes rendelkezések végrehajtásának szabályairól

2009/28/EC Directive on the Promotion of the use of renewable sources and amending and subsequently repealing directives 2001/77/EC and 2003/30/EC

2009/30/EC Directive on regards the specification of petrol diesel and gas-oil and introducing a mechanism to monitor and reduce greenhouse gas emissions

2009/33/EC Directive on the Promotion of clean and energy-efficient road transport vehicles

2010. évi CXVII. törvény a megújuló energia közlekedési célú felhasználásának előmozdításáról és a közlekedésben felhasznált energia üvegházhatású gázkibocsátásának csökkentéséről

No 1316/2013 Regulation (EU) – Establishing the Connecting Europe Facility

2014/94/EU Directive on the deployment of alternative fuels infrastructure

HTTP1: [http://ec.europa.eu/legislation/index\\_en.htm](http://ec.europa.eu/legislation/index_en.htm) (regulations, directives, decisions)

HTTP2: [http://mol.hu/images/pdf/Vallalatiugyfeleknek/Uzemanyagok/Dizel\\_termeklap\\_2011.pdf](http://mol.hu/images/pdf/Vallalatiugyfeleknek/Uzemanyagok/Dizel_termeklap_2011.pdf)

## WATER REGIME SIMULATION ALONG GABČÍKOVO – TOPOĽNÍKY CHANNEL (VRAKÚŇ JUNCTION) CASE STUDY

Petr DUŠEK – Yveta VELÍSKOVÁ – Renáta DULOVIČOVÁ

Institute of Hydrology, Slovak Academy of Sciences, Račianska 75, 831 02, Bratislava, Slovakia,  
E-mail: dusek@uh.savba.sk, veliskova@uh.savba.sk, dulovicova@uh.savba.sk

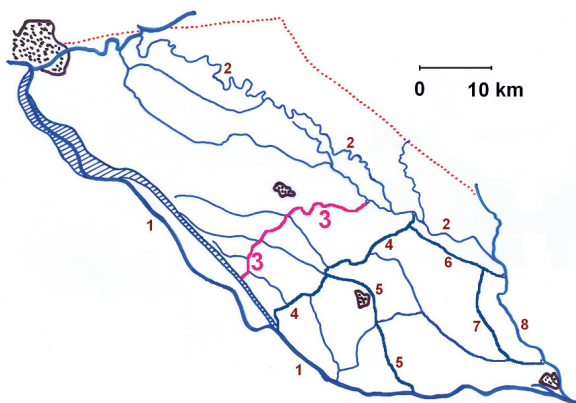
**Abstract:** Gabčíkovo – Topoľníky channel is one of three main channels in the irrigation and drainage channel network of Žitný ostrov. Žitný ostrov is a lowland area in south – western Slovakia with high degree of agricultural use and thanks to its bed composed mainly of fluvial gravel sediments it is a significant storage of high quality groundwater. The Vrakúň junction location was chosen due to the presence of regulation measures (floodgates) along the boundary of the area of interest and also due to the occurrence of both agricultural and urban areas. The aim of this paper is to evaluate surface water – groundwater interactions between the channel and its surroundings considering the significant increase in volume of silts in the channel over the past years and comparison of the interaction between the current state and state with no aggradation in the channel. The simulation was done using finite – difference groundwater (MODFLOW) (Harbaugh et al., 2000) model in the Aquaveo GMS interface.

**Keywords:** surface water, groundwater, interaction, groundwater modeling, MODFLOW

### Introduction

Žitný ostrov is an important agricultural site and it is also an area with important source of drinking water. Last but not least due to the flat nature of the area and built channel network it is the ideal location for exploring the interaction of surface water and groundwater. The aim of this paper is to evaluate the effect of channel network aggradation on the groundwater regime for different scenarios of sediment thickness and its permeability, using one layer finite – difference groundwater flow model.

*Figure 1.* Žitný ostrov (1 – Danube, 2 – Small Danube, 8 – Váh), channel network of Žitný ostrov (3 – Gabčíkovo – Topoľníky channel, 4 – Chotárny channel, 5 – Čalovo – Holiare – Kosihy channel, 6 – Aszód – Čergov channel, 7 – Čergov – Komárno channel, 9 – Komárňanský channel) (Velísková, Dulovičová, 2008)



### Materials and methods

Žitný ostrov is located in the southwestern part of Slovakia, on the border with Hungary. Located in the Danube Lowland, its boundaries are formed to the south by the river of Danube, in the north by Small Danube and the short section on the east it is bordered by the river Váh (Fig. 1).

The average slope of the lowland is only about  $2.54 \cdot 10^{-4}$  (Velísková, Dulovičová, 2008). The case study area is located in the middle of Žitný ostrov near the city of Dunajská Streda. The total area of the modeled locality is approximately 41 square kilometers. The range of altitude is between 112.3 and 115.5 m a.s.l. The terrain level decreases from west to east. From the geological view the area is characterized by gravel or sandy gravel fluvial sediments of significant thickness, ranging from 350 to 400 m in the case study area (Maglay et al., 2009), covered with layer of sand or sandy loam of average thickness 3 – 5 m, and the top layer is composed by loam with average thickness 1 – 2 m (Gyalokay, 1972). Area of interest falls within the climate zone A1, which is characterized as warm, dry, with mild winters and longer sunshine (Faško, Štastný, 2002). The annual precipitation, according to precipitation measuring station,

was 519.3 mm in year 2012 and 670 mm in year 2013. The groundwater recharge is affected mainly by precipitation and also by the seepage from the Danube River.

#### *Model boundaries*

There are five channels from Žitný ostrov channel network present in this area. On the north boundary there are channels Vojka – Kračany (west) and Bohel'ov channel (east), on the south boundary there are channels Šulany – Jurová (west) and Jurová – Veľký Meder (east). The main channel Gabčíkovo – Topoľníky flows through the center of the modeled area (rkm 16 - 24). The area boundary on west and east side (red) was set to specified head as a Time – Variant Specified Head (CHD) boundary (Aquaveo, 2013), set to an approximation of a head value in said area. All of the channels were set up as a river boundary using MODFLOW RIV package (Aquaveo, 2013). The area cells set up with river boundary condition are specified by head stage in river (water level), bottom elevation and a conductance value of the bed sediments. The conductance values of bed sediments were adjusted according to field measurements from channel Gabčíkovo – Topoľníky in 2014.

#### *Topography and geology*

The topography of the area of interest is specified, as with the whole Žitný ostrov, as a lowland area with low slope and small differences between elevations above sea level. Elevations range from 112.3 m a.s.l. to 115.5 m a.s.l (Fig. 2). The width of the model area is approximately 8.7 kilometers and the height of the model area is approximately 7.5 kilometers.

The geology of model area is represented mainly by quaternary fluvial sediments. The area is located in a Gabčíkovo depression, where the thickness of the sediments layer is highest from all of Žitný ostrov. The thickness of the quaternary fluvial sediments ranges from 350 to 400 meters and the depth ranges from -237 meters a.s.l. to -288 meters a.s.l. (Fig. 3).

The hydraulic conductivity of the one layer model geology is represented by a map of horizontal hydraulic conductivities for said area specified in the geological map of Slovakia (Káčer et al., 2005). Saturated hydraulic conductivity of subsurface geology ranges from  $4.2 \cdot 10^{-3}$  to  $8.6 \cdot 10^{-4}$  m.s<sup>-1</sup>.

Figure 2. Topography of model area

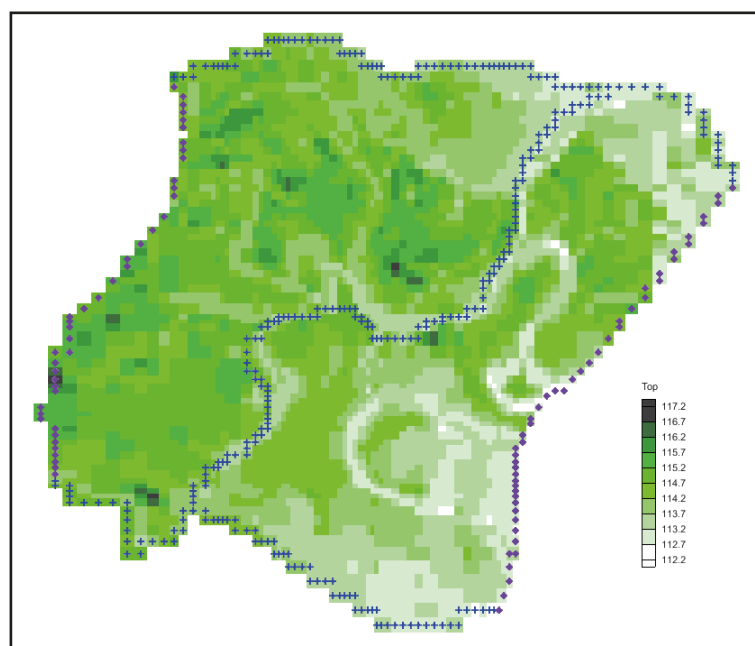
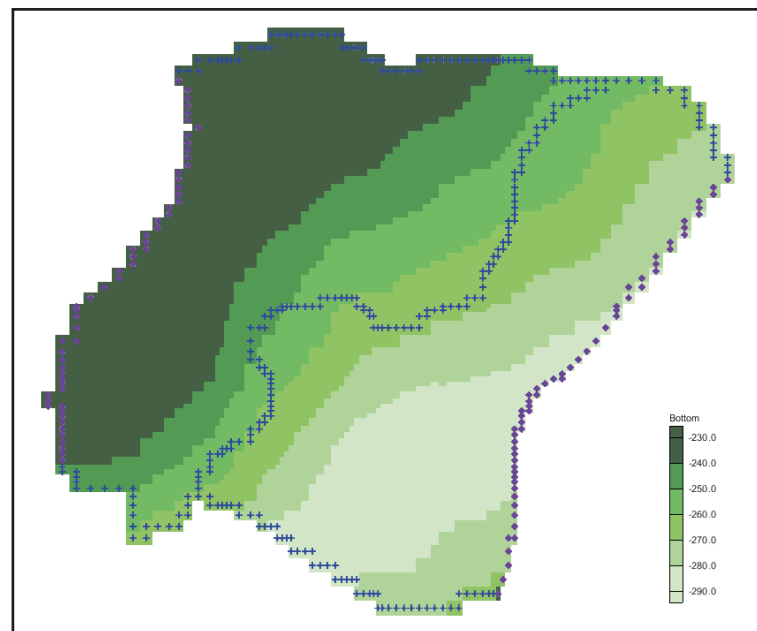


Figure 3. Aquifer bottom depth

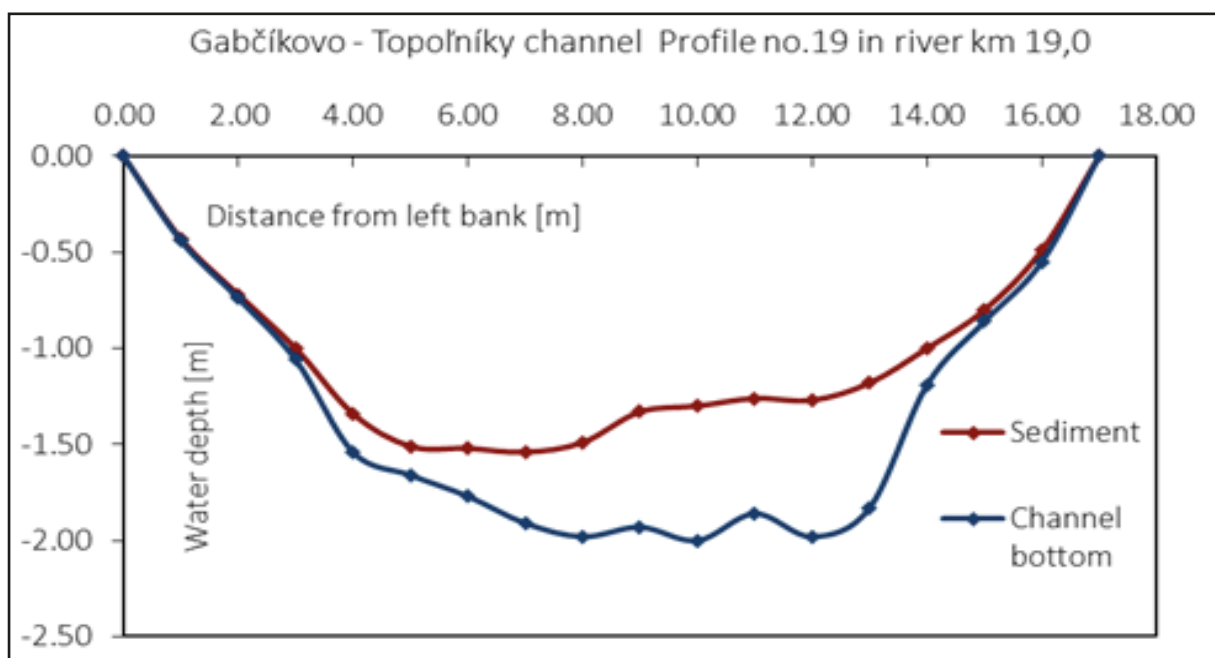


*Channel aggradation*

Complex measurements of Gabčíkovo – Topoľníky channel sediments were completed in May/June 2014 and represent the current state of the channel aggradation. Measurements were done at each river kilometer. Water level and riverbed sediment thickness were measured in each meter across the measured cross-section profile (Fig. 4).

If it was possible sediment samples were taken. In this case study we focused on the rkm 16 – 24, which corresponds to the model area. The sediment thickness ranged from 0.09 m to 0.74 m, with an average of 0.43 m. The granular composition of the samples was evaluated and consequently the hydraulic conductivity was calculated using applicable empirical formulas by Bayer – Schweiger and Špaček (Dulovičová and Velísková, 2005).

Figure 4. Example cross section – thickness of riverbed sediments

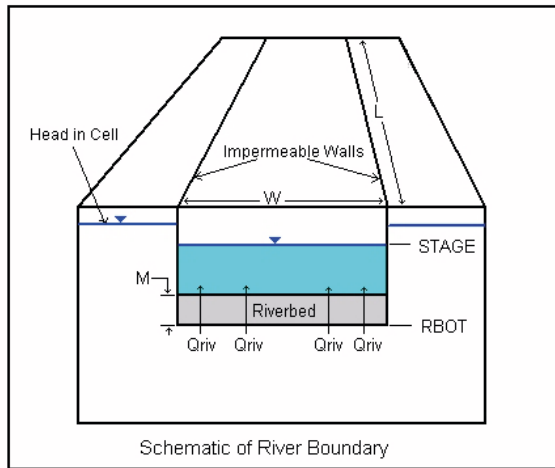


The conductivity values, riverbed thickness and channel width, resp. cross – sectional area were used to calculate conductance, an input parameter of MODFLOW RIV package (Aquaveo, 2013), using following equation:

$$C = \frac{k}{t} lw$$

where  $k$  is hydraulic conductivity,  $t$  is the thickness of the riverbed sediment,  $lw$  is the cross – sectional area perpendicular to the flow direction, and  $L$  is the length of flow (Fig. 5).

Figure 5. Schematization of MODFLOW river boundary (Aquaveo, 2013)



Surface water model

Water level in rivers, in this case in the channels, is an influential factor regarding the simulation of surface water – groundwater interaction. We used a widely used one dimensional surface water simulation package HEC – RAS (U.S. Army Corps of Engineers, 2013) to determine the surface water table elevations throughout the length of the channels based on discharge data from measurement stations at the lower and upper side of the model at the Gabčíkovo – Topoľníky channel and on the channel morphology data obtained by the measurements on site. The output values of water surface elevations were then used as an input parameter of the river package of the

MODFLOW simulation.

Model calibration

Calibration of model was done manually adjusting input parameters of the model (water levels in channels, recharge, hydraulic conductivity) to achieve the best fit of computed and observed head stages in monitoring wells. There are three monitoring wells present in the model area, the observed values were provided by Slovak Hydrometeorological Institute. The model was calibrated to values from May 2014, when the field measurements of riverbed morphology took place on the channel Gabčíkovo – Topoľníky. The maximum difference (error) of simulated vs. observed heads was 0,086 m.

Results and discussion

The first scenario represents the model calibration results (Fig. 6) with current state of sediment deposits. The values of conductance set to each river kilometer ranged from 2.4 to 42.4 m<sup>2</sup>.d<sup>-1</sup>.m<sup>-1</sup>. It shows a W – E groundwater head direction, which corresponds with the general groundwater flow direction at Žitný ostrov. The groundwater heads range from 111.6 m a.s.l on the east to 113.4 m a.s.l on the west. From the direction of the contour lines

Figure 6. Scenario 1 – current state of sediment deposits

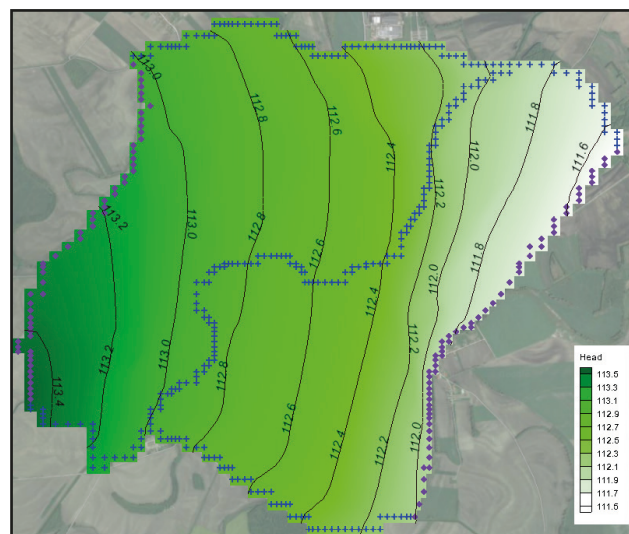


Figure 7. Scenario 2 – significantly more permeable sediment deposits

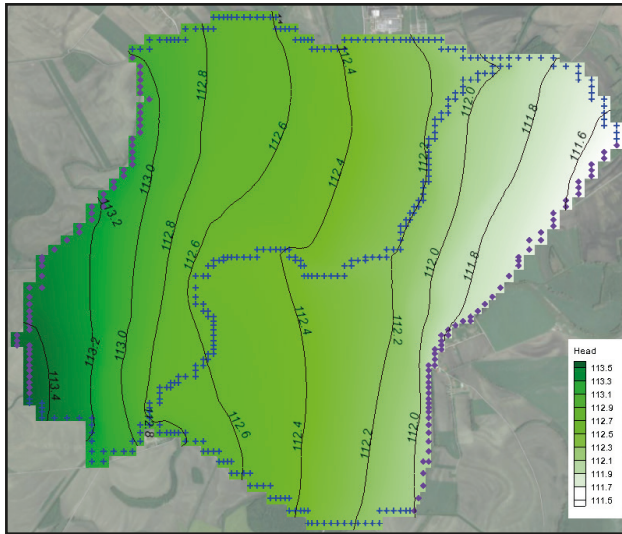
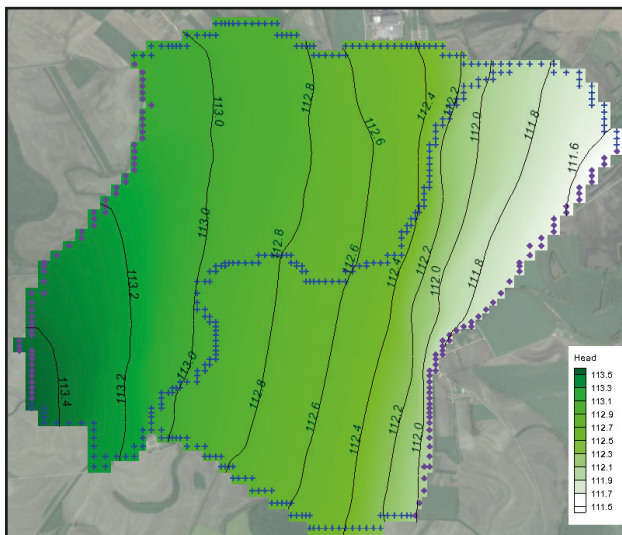


Figure 8. Scenario 3 – less permeable sediment deposits



we can assume that the main channel has less effect on the groundwater level and direction.

The second scenario (Fig. 7) considers all of the channels to be considerably more permeable in terms of bed sediments. All channels were set to a value of conductance of  $500 \text{ m}^2 \cdot \text{d}^{-1} \cdot \text{m}^{-1}$ . The groundwater level in the main channel area drops by 0.2 – 0.4 m. The contour lines

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show a more connected main channel, that is affecting the groundwater direction and flow in the aquifer. In the third scenario (Fig. 8) the channels bottom sediments are considered to be even less permeable than those in first scenario. All channels were set to have conductance of  $3 \text{ m}^2 \cdot \text{d}^{-1} \cdot \text{m}^{-1}$ . The groundwater level raises by additional 0.2 m compared to the first scenario.

## Conclusions

The results show that the hydraulic conductivity and thickness of riverbed sediments in lowland area can significantly affect the water regime and especially the groundwater level. The difference in groundwater table elevations between the current state of the volume and distribution of channel bottom sediments and proposed theoretical scenario of channel without bottom sediments is significant, up to 0.4 meters. Therefore it is important to invest to proper maintenance, because channels clogged with sediments are less effective in the ability to drain excessive groundwater from the aquifer, what is one of the main reasons why the channel network was built. In the occurrence of floods the surrounding agricultural areas are prone to flooding and therefore can be damaged.

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## PERFORMANCE OF WHEAT QUALITY CHARACTERISTICS IMPACTED BY PRECROP AND PLANT NUTRITION

Gergely. SZILÁGYI

University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Crop Science, 4032 Debrecen Böszörményi út 138. E-mail: peszere@gmail.com

**Abstract:** Our field researches took place on the test farm of KIT Látókép of University of Debrecen, Centre for Agricultural and Applied Economic Sciences, in growing seasons 2012/2013 and 2013/2014, after previous cropping of sunflower with control, N<sub>60</sub>+PK and N<sub>120</sub>+PK fertilizer treatments, with varieties Mv Csárdás and Mv Toldi, on chernozem soil, in long-term experiment. According to our data, variety and the effect of the crop year strongly affected the values of the leaf area index (LAI) and the yield. Due to the mild winter in growing season 2013/2014 varieties had much larger assimilative surface, which caused increase in LAI and in the yield. Therefore the natural nutrient utilization capability of the varieties significantly increased. Compared to the previous year, in control treatment we measured 250.0-270.2% higher yield due to the favourable effect of the crop year. Maximum yield (8.21 t ha<sup>-1</sup>) was measured with N<sub>60</sub>+PK fertilizer treatment with variety Mv Toldi. According to our results, in the more favourable crop year of 2013/2014, differences of the SPAD values between the N<sub>60</sub>+PK and N<sub>120</sub>+PK treatments were minimum (changed between 2.8-6.3 units). It means that the effect of fertilization on SPAD values has reached its optimum level according to our yield results.

**Keywords:** winter wheat, crop rotation, nutrient supply, LAI, SPAD, yield

### Introduction

Winter wheat is one of our most important cereal crops. According to Slafer et al. (1994), winter wheat is one of the most widely grown cereal plant, produced in all regions of the world. According to Rajaram and Braun (2008), by 2020, 840-1000 M tonnes of winter wheat will be utilized. According to Richards (2000), compared to the previous century, yield of winter wheat has doubled. As per the data of the Central Statistics Office (2014), in our country the national average yield was 4.64 t ha<sup>-1</sup> in 2013 and 4.64 t ha<sup>-1</sup> in 2014. The crop year, the crop rotation and the nutrient supply affect its production the most extent. According to Pepó and Csajbók (2014), the effect of fertilization is less (26-28%) in crop years with favourable water supply, and more significant (50-75%) in dryer crop years. The second most important factor that affects yield is crop rotation 10.38-37.75%, depending on the crop year. Crop structure of our country has changed in a disadvantageous way, rate of cereal and oil crops has been centralized. Jolánkai et al. (2013) claimed that the negative effect of monoculture was also measurable

from the 6<sup>th</sup> year of continuous cropping. According to Berzsenyi and Danq (2002), nitrogen fertilization has a positive effect on the vegetative development of winter wheat. According to the research results of Lönhardné and Kismányoky (1992), the nitrogen significantly increased leaf coverage, leaf area duration and plant height. The leaf area index was first defined by Watson (1947), who compared the measured leaf area with a unit area of the soil.

According to Yang et al. (2007), the leaf area index (LAI) is an important parameter in such models which are related to the ecosystem, the carbon management, the climate and hydrology. Changing of the LAI values is an important point during production. According to Pepó (2005), maximum values of LAI were produced in the period of coming into ear-flowering in both crop years. In the dry year 2003, with N<sub>120</sub>+PK treatment, 3.31-3.62 m<sup>2</sup>m<sup>-2</sup>, while in 2004 with favourable weather 6.91 – 7.66 m<sup>2</sup>m<sup>-2</sup> maximum LAI values were measured. Salvagiotti and Miralles (2008) partly agrees with Pepó's conclusions (2005), according to their results

they measured maximum LAI values up to 9.46 m<sup>2</sup>m<sup>-2</sup>. According to the results of Sugár and Berzsenyi (2009), in 2007 outstanding LAI values (10.6 – 11.5 m<sup>2</sup>m<sup>-2</sup>) were observed with variety Mv Toborzó with N<sub>160</sub> and N<sub>240</sub> fertilizer treatments. According to Kulig et al. (2010), LAI values correlate to the yield, the crop quality, the thousand grain weight as well as to the number of ears. Global warming affects plant production of our country, too. According to Tian et al. (2012), as an effect of warming, the flag leaf area increased by 36%, in the phenophase of flowering the total leaf area increased by 19.2%, which resulted in the increase of the yield.

Winter wheat can be rated to the group of C3 plants, the extent and volume of nutrient usage depend on the chlorophyll content. According to Wood et al. (1993), the Minolta SPAD appliance is a small manual spectrometer that measures the adsorption of light (650 nm) on the surface of the leaf, which is a non-destructive gauge of the chlorophyll and nitrogen content of the plant. According to Markwell et al. (1995) measurement of the chlorophyll content with Minolta SPAD-502 is appropriate. His research results are confirmed by that according to Ommen et al. (1999, there is a strong significant correlation between the SPAD values and the total chlorophyll content. Monje and Bugbee (1992) stated the opposite saying that the relationship between the chlorophyll content and the values measured by the Minolta 502 appliance is not linear. Fekete et al. (2015) claimed that the doses of nitrogen applied have significant impact on the SPAD values. According to Szilágyi (2014), with genotype Mv Csárdás, maximum SPAD value (42.8) was observed with N<sub>120</sub>+PK fertilizer treatment –after sunflower previous cropping - , in latic ripeness (BBCH 73-77). According to his data, there are considerable differences between the varieties. As for differences between the fertilizer treatments, according to Shi et al. (2010), there was no difference between SPAD values measured in N<sub>130</sub> and N<sub>300</sub> kg ha<sup>-1</sup> treatments.

## Material and method

Our researches done in long-term experiment took place on the test farm of KIT Látókép of University of Debrecen, Centre for Agricultural and Applied Economic Sciences, on calcareous chernozem soil in growing seasons 2012/2013 and 2013/2014. We examined to different genotypes (Mv Csárdás and Mv Toldi) after previous cropping of sunflower. The experiment were arranged in 4 replicates in split bands. We examined three different nutrient level. Besides the control treatment we spread dosages of N=60 kg ha<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub>=45 kg ha<sup>-1</sup> and K<sub>2</sub>O=53 kg ha<sup>-1</sup>; N=120 kg ha<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub>=90 kg ha<sup>-1</sup> and K<sub>2</sub>O=106 kg ha<sup>-1</sup>. We spread the nitrogen in 50-50% autumn and spring split, and spread 100% of the phosphorus and potassium dosages in autumn (Table 1.).

Table 1. Applied fertilizer doses (Debrecen, 2013-2014)

Fertilizer treatment	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
	kg ha <sup>-1</sup>		
Control	0	0	0
N <sub>60</sub> +PK	60	45	53
N <sub>120</sub> +PK	120	90	106

As per our examinations, growing seasons 2012/2013 and 2013/2014 can be considered as favourable, based on the measured meteorological data. According to the precipitation data, in the growing season 2012/2013, 480.2 mm, and in growing season 2013/2014 284 mm of rain fell. In the growing season 2013/2014, the average temperatures of 2.0 °C in January and 3.5 °C in February were extremely mild, which helped the development of winter wheat. We determined the LAI values with SunScan Canopy Analysis Systems (SS1) meter. SPAD results were defined with Minolta 502 hand machine, based on BBCH scale. According to phenological states of development, we carried out our measurements at stem elongation (BBCH 30-39) in stage with flowering (BBCH 61-69) during the stage of latic ripeness (BBCH 73-77) and grain ripening (BBCH 83-89). We averaged the LAI and SPAD values. We

measured our yield results with Sampoo parcel harvester. Our research data were analyzed by Microsoft Excel program. Our results demonstrate correlations between LAI and yield in growing seasons 2012/2013-2013/2014.

## Results

LAI and SPAD values measured in the examined growing seasons are shown in Figure 1. As an effect of fertilization, LAI values of the examined varieties changed on the same extent, no difference was observed between them. During linear regression analysis, close correlation was detected when analysing the LAI values of the varieties (Mv Csárdás  $R^2 = 0.9601 - 0.9908$ , Mv Toldi  $R^2 = 0.9700 - 0.9845$ ). In growing season 2013/2014, a large difference was measured during the analysis of the LAI and the yield (Table 2).

to our results, in the more favourable crop year of 2013/2014, differences between SPAD values measured with  $N_{60}+PK$  and  $N_{120}+PK$  treatments changed between 2.8-6.3 units. It indicates that the effect of fertilizer treatments on SPAD values has reached the optimum level. According to our data (Figure 2.), in crop year of 2013/2014, with the tested varieties, maximum yields were measured with the  $N_{60}+PK$  treatment (Mv Csárdás  $6.27 \text{ t ha}^{-1}$  – Mv Toldi  $8.28 \text{ t ha}^{-1}$ ).

Our data show that in the more favourable crop year (2013/2014) a larger difference (+23.1, +25.1) was detected between the control and the  $N_{120}+PK$  treatments, than in crop year 2012/2013 (+15.9, +18.7).

Based on the data of the analysed years, after previous cropping of sunflower, yield of winter wheat was influenced by the crop year and the

Table 2. Polynomial regression (yield (2013/2014)) and linear regression (LAI – SPAD – yield (2012/2013)) equations (Debrecen, 2013-2014)

Genotypes	Mv Csárdás	Mv Toldi
SPAD (2013. year)	$y = 9,35x + 16,933 \text{ R}^2 = 0,9794$	$y = 7,95x + 16,567 \text{ R}^2 = 0,9693$
SPAD (2014. year)	$y = 11,55x + 14,3 \text{ R}^2 = 0,9356$	$y = 12,55x + 13,5 \text{ R}^2 = 0,8325$
LAI $\text{m}^2\text{m}^{-2}$ (2013.year)	$y = 1,2x - 0,5333 \text{ R}^2 = 0,9908$	$y = 1,15x - 0,4667 \text{ R}^2 = 0,9845$
LAI $\text{m}^2\text{m}^{-2}$ (2014.year)	$y = 1,7x - 0,1 \text{ R}^2 = 0,9601$	$y = 2,3x - 0,5667 \text{ R}^2 = 0,9700$
Yield (2013. year)	$y = -0,61x^2 + 4,32x - 2,3 \text{ R}^2 = 1$	$y = -0,27x^2 + 3,01x - 1,14 \text{ R}^2 = 1$
Yield (2014. year)	$y = -1,375x^2 + 6,585x - 1,4 \text{ R}^2 = 1$	$y = -2,22x^2 + 10,94x - 4,72 \text{ R}^2 = 1$

Considering the SPAD values, in growing season 2012/2013, small differences were observed between the genotypes. As an effect of higher fertilizer treatment, compared to the control treatment, SPAD values of each varieties were higher (10.4-11.7 units) with  $N_{60}+PK$  treatment. Differences between the SPAD values measured with  $N_{60}+PK$  and  $N_{120}+PK$  treatments changed in a narrow interval (5.5-7.0 units)

Differences between those values measured with control and increased fertilizer treatments changed between 15.9-18.7 units. According

genotype. In growing season 2012/2013, the yield changed in nearly the same interval in case of all the nutrient treatments with the examined varieties (Mv Csárdás  $1.41-5.17 \text{ t ha}^{-1}$  – Mv Toldi  $1.60 - 5.46 \text{ t ha}^{-1}$ ). During the analysis of the yield we observed that the varieties utilized the naturally available nutrients of the soil on the same extent (Mv Csárdás  $3.81 - \text{Mv Toldi } 4.00 \text{ t ha}^{-1}$ ). We measured a much higher increase (250.0-270.2%) in the yield compared to the previous growing season in control treatment. Maximum yield was reached with  $N_{60}+PK$  treatment, with variety Mv Toldi. Maximum yield of Mv Csárdás was lower by  $2.01 \text{ t ha}^{-1}$

Figure 1: Average LAI and SPAD values after previous cropping of sunflower at different fertilizer dosages (Debrecen, 2012/2013-2013/2014)

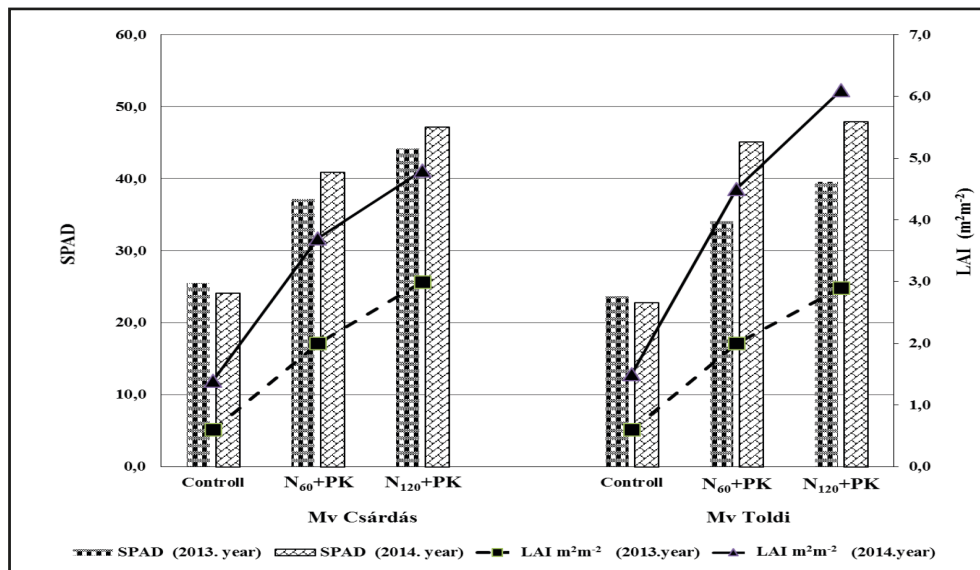
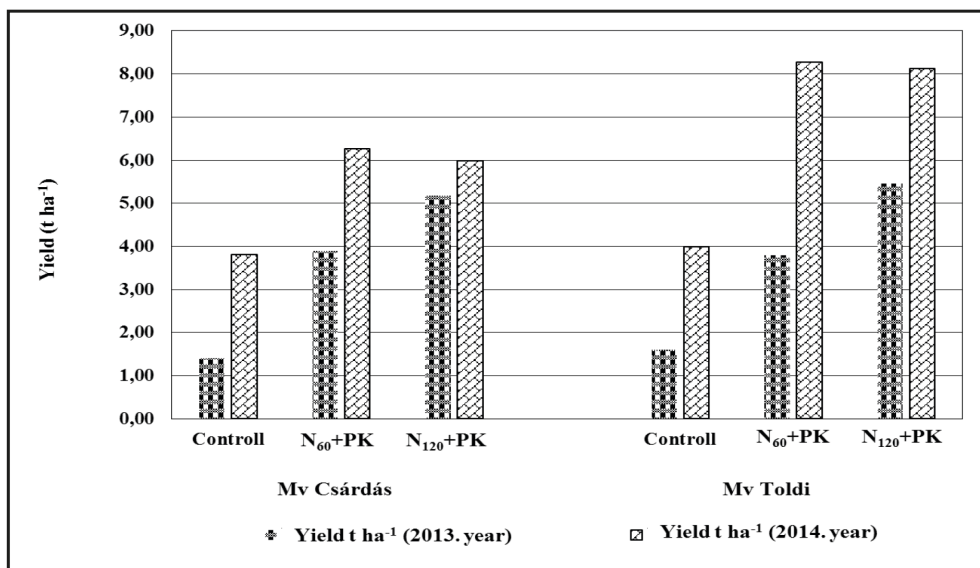


Figure 2. Yield results after previous cropping of sunflower at different fertilizer dosages (Debrecen, 2012/2013-2013/2014)



than with genotype Mv Toldi. According to our results, in growing season 2013/2014 the yield and the LAI values in N<sub>60</sub>+PK and N<sub>120</sub>+PK treatment were much higher than in the previous growing season. During regression analysis, we detected close correlation when we analysed the LAI values ( $R^2 = 0.9601 - 0.9908$ ) and the yields ( $R^2 = 1$ ). Results of our polynomial regression analysis show that there is a close correlation between the SPAD  $R^2$  values of the varieties in the examined years (Mv Csárdás 0.9356 – 0.9794; Mv Toldi 0.8325 – 0.9693).

## Discussion

According to the data of our field researches, the effect of the crop year significantly influenced the leaf area index (LAI), the chlorophyll content (SPAD) and the yield of winter wheat after previous cropping of sunflower. Based on our plant physiological measurements, during the tests of the varieties, we observed considerable differences. Our researches confirm research results of Pepó (2005), according to which significant

differences between the varieties were due to adaptation reasons. Our data confirm the results of Tian et al. (2012): with the increase of the average temperatures of winter months, the varieties reached higher LAI values and yield. This means that the genotypes utilize the naturally available nutrients of the soil and the applied fertilizer dosages also in calcareous chernozem soil, in case of a mild winter. Our results confirm the results of Markwell et al. (1995) and Fekete et al. (2015) also.

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### **Source of the graphics**

*Front cover:*

Gallo-Roman harvesting machine, called Vallus. Source: U. Troitzsch - W. Weber  
(1987): Die Technik : Von den Anfängen 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



### **POSTA Katalin, editor-in-chief**

DSc /agric/, dean of the the Faculty of Agricultural and Environmental Sciences of the Szent István University, Gödöllő, Hungary, member of the Soil Science, Water Management and Crop Production Committee of the Hungarian Academy of Sciences. Professional fields: soil microbiology, arbuscular mycorrhizal fungi, plant protecton by soil microorganisms.



### **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 19<sup>th</sup> Century.