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Editorial

Science and environment – agriculture and environment

The most important goal of agricultural production is to deliver healthy, sufficiently nutritious and residue-free food raw material. Similarly to any economic activities, producing crops and agricultural goods is expected to be both economic and affordable for the consumer. In accordance with the aims of those engaged in agriculture, this activity is carried out by maintaining the fertility of soil, i.e. in a sustainable manner. One of the most crucial tasks of scientific research focusing on agricultural production is to support and promote this goal.

Production, however, heavily affects the environment. The accumulation of contaminants in soil or in surface waters and ground waters, and thus the environmental pressure of agricultural activities, is a major challenge for both the production and the scientific research underlying and promoting it. Sustainability of production can only be assessed in this broader aspect, and this is also the only way to ensure the protection of consumers' health.

We trust that the scientific results published in Columella will help to achieve the abovementioned goals of agricultural production, and that science can thus also contribute to environmental sustainability.

Agricultural ecosystems are Hungary's most extensive human dominated habitats. These areas are home not only to attacking pests and vermin, but also to a range of valuable protected and game species, as agricultural production also affects protected areas and hunting grounds.

Species occurring in agricultural habitats and their population dynamics can be rightly considered as specific indicators of the quality of production. The status of a population of any species is primarily determined by the quality of the habitat. This means that if the densities of the indicator species is stable or growing, it suggests high-quality habitats and thus good agricultural management. When, however, this number is dropping, it can indicate that something might be going in a wrong way. But what does it mean when the population of certain species (e.g. brown hare, pheasant, grey partridge) is decreasing, whereas that of others (e.g. greylag goose, roe deer, common wood pigeon) is rising?

Is our agricultural management good or bad? Does science serve agriculture and environment well or not? Are we capable of changes or providing alternatives in this field? Do we know what to change, what to alter at all? And if we do, can we explain how to utilise that in practice? So can we teach what we all need for the sake of our future? This is the responsibility of science and education for our environment and for our future. It is my earnest wish that Columella and its publisher, the Faculty of Agricultural and Environmental Sciences may serve this purpose well.

Gödöllő, 2nd. June 2019

Miklós Heltai editor-in-chief

The role of GIGANTEA in flowering and abiotic stress adaptation in plants

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Abstract: GIGANTEA (GI) is a clock-regulated, nuclear-localised plant protein. It invaluably contributes as a core element with pleiotropic functions in the cardinal plant physiological pathways including flowering time regulation, circadian clock control, abiotic stress tolerance, and miRNA processing. This review aims to highlight the importance of GI and elucidate on the participatory mechanism it follows to regulate plant responses. An attempt is made to concisely present the pivotal functions of GI in *Arabidopsis* drawing an analogy with the functions of the paralogs in other species underlining its conserved nature. This paper also strives to draw attention to the possibility of considering *GI* as a candidate gene for modulation to enhance tolerance against abiotic stresses.

Keywords: GIGANTEA, flowering time regulation, circadian clock control, GI orthologs, abiotic stress adaptation

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Introduction

Several abiotic factors have been hindering agricultural production by affecting the stages of germination, vegetative and reproductive growth stages (Zhu, 2002; Sivakumar et al., 2005; Rengasamy, 2010; Lobell and Gourdji, 2012). The embolisms resulting from the restraining environmental conditions amend the plants' ability to combat the stress and acclimatize within the prevalent conditions for instance by conserving water under water deficit conditions (Chaves et al., 2003). One of the many methods to achieve the ultimate goal of sustainable crop production is genetic modification using known abiotic stressrelated genes from other species or precise gene identification of the plants and upregulating or down-regulating existing genes to either escape or tolerate adverse conditions by harnessing the plants' own defence mechanisms (McKay et al., 2003; Kim et al., 2011; Verslues and Juenger, 2011; Tao et al., 2015; Ke et al., 2017). Plants are inherently designed to evaluate the environment around them and resume growth when the conditions are in their favour (Zeevaart, 2006). They measure variables such as day length and temperature to transform to flowering stages followed by reproduction under normal conditions and thereby adapt to the naturally occurring fluctuations gradually by their system of signalling pathways (Jung and Müller,

2009; Sawa and Kay, 2011). The flowering pathway could follow three directional effectors: photoperiod, vernalisation (cold) and autonomous (endogenous factors as hormones) effectors to modulate flowering as a response to environmental cues (McClung, 2006; Andrés and Coupland, 2012; Song et al., 2015; Bouché et al., 2017; Cheng et al., 2017).

Effect of photoperiod on flowering

Photoperiodism, which refers to the rhythms of biological processes that are based on daylength changes, is one of the most stressed parameters due to its cyclic periodicity and dependability that governs the transitions in crop growth. The duration of daylight is measured in the photoperiodic flowering pathway by CONSTANS (CO), which is a B-box-type zinc finger protein that shares identity with GATA transcription factors (Samach et al., 2000; Suarez-Lopez et al., 2001; Yanovsky and Kay, 2002; Imaizumi and Kay, 2006; Corbesier and Coupland, 2006). The stability of CO protein is regulated by light and under long day conditions (LD) (16 h of light and 8 h of darkness) it activates florigen genes, which are peptide hormones genes, and TWIN SISTER OF FT (TSF) in the phloem companion cells (An et al., 2004; Valverde et al., 2004; Yamaguchi et al., 2005; Jang et al., 2009). It then progresses towards

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shoot apical meristem (SAM) and activates the FLOWERING LOCUS T (FT) inducing accelerated flowering (Valverde et al., 2004; Abe et al., 2005; Wigge et al., 2005; Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007). Under short day conditions (SD) (8 h of light and 16 h of darkness), the peak time of CO expression occurs after dusk rendering the CO protein unstable and resulting in incongruent activation of FT (Yanovsky and Kay, 2002; Valverde et al., 2004). Thus the timing of CO expression is a cardinal factor in the photoperiodic flowering pathway which is under the influence of several associated genes and interactions which eventually send signals to the SAM to shift from vegetative to reproductive stage (Bernier et al., 1993). Several transcription factors constituting the circadian clock ensure the systemic functioning of the central signal pathway and control not only flowering but also the rhythmic expression of abiotic stress-responsive genes (Grundy et al., 2015). One such closely associated gene with the circadian clock functioning is *GI* (Takada and Goto, 2003).

Latitudinal gradient influences *GI* expression by providing varying day lengths and in turn varying photoperiods to respond to. GI being sensitive to longer photoperiods has a delayed expression in *Arabidopsis* accessions originating from varying latitudes and exposed to LD conditions. The rate of change in day length conferred by latitudinal positions also influences *GI* expression and is regulated differently in the northern and equatorial

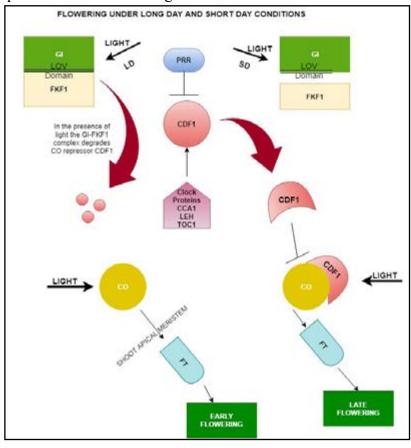


Figure 1. Flowering pathway under long day (LD) and short day (SD) conditions. GI interacts with FKF1 through the Light, Oxygen or Voltage domain (LOV) and forms a complex which then degrades the CONSTANS (CO) repressor CYCLING DOF FACTOR (CDF1). CDF1 is repressed by PSEUDO RESPONSE REGULATOR proteins (PRRs) but is activated by the clock proteins CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LEH) which control GI peaks and negatively regulate the transcription of TIMING OF CAB1 (TOC1), which acts as a negative feedback. The CO then activates FLOWERING LOCUS T (FT) which then induces early flowering under LD and late flowering under SD conditions. Bold arrows indicate activation. Normal arrows indicate transcriptional activation. Perpendicular lines indicate transcriptional repression. The model is based on the publication by Johansson and Staiger (2015).

regions. The changes in *GI* expression impact plant growth rate presumably by regulating *PHYTOCHROME INTERACTING FACTOR* 4 (*PIF4*) expression (de Montaigu and Coupland, 2017).

Effect of GI-FKF1 interaction on flowering

GIs are large plant proteins exclusively belonging to plants and possess several functional domains that can actively influence the signalling pathways such as circadian control by light signalling, flowering, response to abiotic stresses and circadian rhythm (Kim et al., 2013a; Mishra and Panigrahi, 2015). They are required for phytochrome B signalling pathway as an intermediate in the photoperiodic control of flowering. Under LD conditions gi mutants flower comparatively late and under SD conditions they flower earlier than the wild type and the phenotypical changes are characteristic to the reception of red light (Huq et al., 2000). In Arabidopsis, GIs were originally identified due to their contribution to photoperiodic flowering and circadian clock regulation (Fowler et al., 1999; Suarez-Lopez et al., 2001; Martin-Tryon et al., 2007; Mishra and Panigrahi, 2015).

The function of GI in the photoperiodic flowering and in circadian rhythms has been extensively studied from monocot to dicot plants and is observed to have highly conserved functions which involve three negative feedback interlocked cycles: the morning-expressed CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LEH), and the evening-expressed TIMING OF CAB (TOC) (Mouradov et al., 2002; Song et al., 2010; Kim et al., 2012). GIs are predominantly nuclear localised particularly in the nucleoplasm and are also present in the cytosol and many plant tissues including vascular bundles, mesophyll, apical shoot meristem and root (Hug et al., 2000). GI acts in the LD flowering pathway upstream of CO and FT (Tseng et al., 2004). As shown in Figure 1, GI forms a complex with

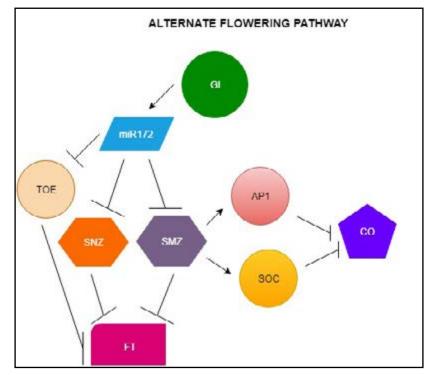


Figure 2. The alternate flowering pathway. GI regulates the amount of miR172 which further interferes with the mRNA of several FT repressors like TARGET OF EAT 1 (TOE1), SCHLAFMUTZE (SMZ) and SCHNARCHZAPFEN (SNZ). SMZ apart from directly repressing FT also regulates APETALA1 (AP1) and SUPRESSOR OF CONSTANS OVEREXPRESSION (SOC1). SOC1 represses CONSTANS (CO) transcription. Arrows indicate transcriptional activation. Perpendicular lines indicate transcriptional repression. The model is based on the publication by Jung et al. (2007).

the FLAVIN-BINDING, KELCH REPEAT, F-BOX 1(FKF1) protein which controls daytime CO transcription in a light-dependent manner by degrading a key CO repressor, CYCLING DOF FACTOR 1 (CDF1) expressed only in the vascular bundles (Fornara et al., 2009). Under LD conditions the expression of GI and FKF1 peaks simultaneously, leading to the optimal formation of the GI-FKF1 complex, and since CO expression is stable, creating an ambient and desirable condition for flowering. Whereas, under SD conditions, the expression of GI peaks before the peak of FKF1 expression by few hours resulting in a lower amount of GI-FKF1 complex. In turn, the degradation of CDF1 is disrupted (Sawa et al., 2007, 2008).

Effect of GI-miR172 interaction on flowering

Genetic analysis of the flowering pathway has suggested an alternate pathway for flowering which could be merging into the CO-FT pathway or could be possibly running individually and is regulated by GI (Mizoguchi et al., 2005). It was reported that GI is capable of regulating FT expression independent of CO by interfering with miR172 levels (Mizoguchi et al., 2005; Jung et al., 2007) as depicted in Figure 2. As the transcriptional factors targeted by miR172 actively partake in flowering such as TARGET OF EAT (TOE1, TOE2 and TOE3) which is involved in the induction of FT expression, SCHLAFMUTZE (SMZ) and its paralog SCHNARCHZAPFEN (SNZ) which represses FT, it makes the GI-miR172 interaction, where GI influences the amount of miR172, as one of the interesting facets in regulating flowering (Jung et al. 2007; Mathieu et al., 2009). Beside the repression of FT, SMZ also regulates the expression of APETALA1 (API) and SUPRESSOR OF CONSTANS OVEREXPRESSION (SOC1), which regulate flowering time and floral development in SAM bolstering the importance of GI in the flowering pathway (Mathieu et al., 2009).

Unlike *CO* repressor CDF, several *FT* repressors like FLOWERING LOCUS C (FLC), SHORT VEGETATIVE PHASE (SVP), TEMPRANILLO (TEM)1 and TEM2

are not limited to the vascular bundles and when GI was expressed ectopically in the mesophyll cells, where CO is absent, it was shown to induce FT expression in the tissue. This finding consolidates the existence of an alternate photoperiodic flowering pathway possibly involving GI independent of CO. The expression of FT in the mesophyll is associated with the fact that GI is capable of binding to the FT repressors at the promoter regions and influencing flowering mostly due to their shared similarities in chromatin-binding pattern (Sawa and Kay, 2011).

Effect of GI-Zeitlupe interaction on flowering

Further partaking in the circadian rhythm, GI interacts with the F-box protein ZEITLUPE (ZTL), which is a blue-light photoreceptor found in the cytosol. As presented in Figure 3, the interaction is through the aminoterminal flavin-binding LIGHT, OXYGEN or VOLTAGE (LOV) domain of ZTL in a direct protein-protein interaction. The immature ZTL is carried by the molecular chaperon HSP70. The interaction between GI and ZTL results in maturing of ZTL facilitated by the chaperon HSP90. The mature ZTL dissociates from the complex (Cha et al., 2017). ZTL maintains a normal circadian period by regulating the proteolytic degradation of the central circadian oscillator, TIMING OF CAB 1 (TOC1) and PSEUDO RESPONSE REGULATOR (PRR5) (Kim et al., 2007). Hence, the GI-ZTL interaction has a strong influence on TOC1 and in turn the circadian clock (Froehlich et al., 2002; Harper et al., 2003; Martin-Tryon et al., 2007; Cha et al., 2017).

Conservation of GI function in flowering

Though the GI gene has gone through many intraspecific gene duplications like the four known paralogs of soybean (GmGI 1a, GmGI 1b, GmGI 2 and GmGI 3), and the two GI-like genes (AcGIa and AcGIb) involved in flowering promotion in onion (Taylor et al., 2010; Watanabe et al., 2011), the functions of the GI seem to be conserved. Poplar being a woody plant differs from Arabidopsis in several ways but in poplar varieties, the

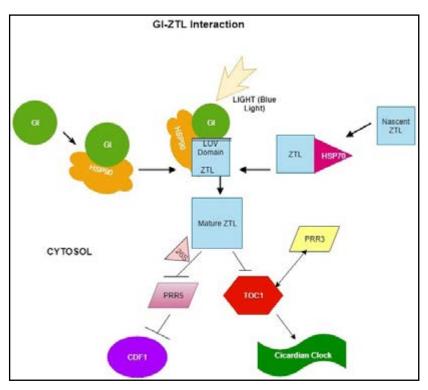


Figure 3. GI-ZTL interaction. GI interacts with Zeitlupe protein via the Light, Oxygen or Voltage (LOV) domain in a protein-protein interaction. HSP90 chaperone carries GI and HSP70 chaperone carries nascent ZTL. The ZTL-GI complex is formed with the help of HSP90 in light. The mature ZTL exits the complex and proteolytically degrades TIMING OF CAB1 (TOC1) and PSEUDO RESPONSE REGULATOR 5 (PRR5), a repressor of CYCLING DOF FACTOR 1 (CDF1). PSEUDO RESPONSE REGULATOR 3 (PRR3) interacts with the N terminus of TOC1 competing with ZTL, therefore during less light and low levels of ZTL, it prevents TOC1 from degradation. Arrows indicate transcriptional activation. Perpendicular lines indicate transcriptional repression and bold arrows indicate the transport and change in conformation. The two-headed arrow depicts protein-protein interaction. The model is based on the publication by Cha et al. (2017).

GI paralogues, PagGIs, are similar in their functions. physiological However, regulation of PagGIs is different (Baurle and Dean, 2006; Jansson and Douglas, 2007; Ke et al., 2017). As in Arabidopsis, PagGIs regulate the circadian rhythms through a protein-protein interaction with the PagZTLs, which is vital for the proteasomal degradation of PagTOC1 (Kim et al., 2007, 2013b). PagGIs also appear to regulate flowering in a similar manner in poplar like in *Arabidopsis* by having an impact on the functioning of the homolog of CO, PagCO2 and progressing through the PagGI-PagCO2-PagFT pathway possibly playing a role in the regulation of both flowering time and the timing of growth cessation (Böhlenius et al., 2006; Ke et al., 2017).

Despite the similarities shared by GI homologues, there is a difference in the pattern of flowering regulation mediated by GI initiation in LD and SD crops. In SD crops such as rice the CO homolog OsHd1

when regulated by OsGI, the GI homolog, inhibited the expression of the FT homolog OsHD3a leading to delayed flowering phenotype (Hayama et al., 2003). Whereas in LD Arabidopsis, GI activates CO under LD conditions and CO further activates FT resulting in blooming. The delayed flowering observed in soybean, maize and morning glory on the overexpression of GI homologs due to down-regulation of FT homologs consolidates the idiosyncrasy of SD crops and LD crops and the difference in the effect of GI expression (Higuchi et al., 2011; Bendix et al., 2013; Li et al., 2013). Sweet potato, an SD crop having the GI gene paralog IbGI, shares more than 70% identity with other GI paralogues AtGI (Arabidopsis thaliana), StGI (Solanum tuberosum), PnGI (Ipomoea nil) and SlGI (Solanum lycopersicum). IbGI is also majorly nuclear-localised and IbGI has evident circadian rhythms with variation under LD and SD conditions. Furthermore, it can restore the AtGI function in *gi-2* mutant (Tang et al., 2017).

StGI and StFKF1, the GI and FKFI orthologues in *Solanum tuberosum*, regulate *StCO1* and *StCO2*. Activity of *StCO* genes repress tuber formation under LD in abundance of StCDF1. StCDF1 down-regulates *StCO1* and *StCO2* and the proteins encoded by them suppress the transcription of the potato *FT* homologue, *StSP5G*, enabling synthesis of the mobile StSP6A signal and resulting in the induction of tuber development at the stolon termini (Kloosterman et al., 2013).

Effect of GI on abiotic stress adaptations

Flowering time alterations are an evolutionary strategy imbibed by plants to maximize the probability of reproduction under varying stress conditions (Kazan and Lyons, 2015)

and the transition occurs when reproduction coincides with suitable external conditions (Andrés and Coupland, 2012; Blümel et al., 2014). Different plants have their own inherent response to external stresses. Varieties within crop species also have varying photoperiod sensitivities generated via environmental adaptations or through breeding (Coles et al., 2010; Gómez-Ariza et al., 2015). As seen in *Figure 4*, GI plays an active role in abiotic stress regulation conferring tolerance to plants under unfavourable conditions.

GI functions in conferring salt tolerance to crops through the Salt Overly Sensitive (SOS) signalling pathway which maintains ion homeostasis conserved in dicot plants such as *Arabidopsis* and *Brassica nigra* (Zhu, 2002; Tang et al., 2015). Under saline conditions, the Na⁺ levels are modulated via three known

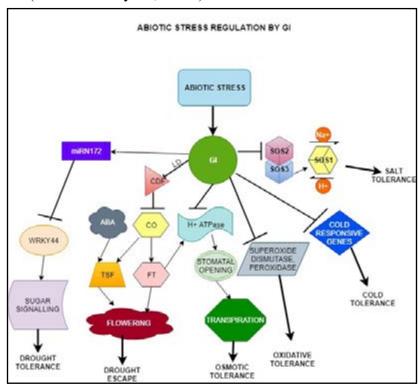


Figure 4. Abiotic stress regulation by GI. GI interacts with the Salt Overly Sensitive SOS2 and SOS3 proteins. Under salt stress conditions, GI undergoes proteolytic degradation, SOS2 phosphorylates SOS3 forming a complex which in turn activates SOS1 to exchange ions and maintain ion homeostasis. GI represses the cold responsive genes. In gi mutants, the cold repressive genes are upregulated conferring cold tolerance to crops, while the higher levels of superoxide dismutase and peroxidase provide tolerance to oxidative stress. GI confers osmotic tolerance by inhibiting stomatal opening regulated by H⁺-ATPase following multiple pathways. GI-CDF-CO-FT is one of the interfering pathways as FT maintains the H⁺-ATPase activity. Under drought stress, the GI represses CDF thereby promoting CO expression which in turn upregulates FT and TSF. ABA also promotes florigen gene expression resulting in early flowering hence drought escape. In addition, GI regulates miR172 levels which represses WRKY44. WRKY44 participates in sugar signalling which eventually brings about drought tolerance. Arrows represent activation. Perpendicular lines indicate inhibition. Bold arrows indicate the impact. The model is based on the publication by Kazan and Lyons (2015).

constituents: calcium-binding protein SOS3, protein kinase SOS2 and plasma membrane Na⁺/H⁺ antiporter SOS1. GI contributes to the pathway by binding to SOS2 kinase and preventing the phosphorylation that occurs between SOS2 and SOS3 thereby interfering with the activation of SOS1 under normal conditions (Halfter et al., 2000; Guo et al., 2001; Ji et al., 2013; Kim et al., 2013a). However, in the presence of high salt, GI undergoes proteasomal degradation by 26S and the unbound SOS2 interacts with SOS3 to form an active SOS2-SOS3 protein kinase complex, which subsequently activates the plasma membrane localised Na⁺/H⁺ antiporter SOS1. As a result, sodium ions are exported from the cell and salt tolerance is established (Kim et al., 2013a).

Drought arrests floral development and induces sterility (Su et al., 2013). Water availability impacts flowering time and to escape drought period many plants are observed to accelerate their flowering (Franks, 2011). With respect to drought escape, GI seems to have a prominent role in regulating plant response. During LD, drought stress incites induction of FT and TSF in a GI-regulated pathway whereas under SD, floral repressors are activated (Riboni et al., 2013). The phytohormone abscisic acid (ABA) is also required for the drought escape response, by promoting the transcriptional up-regulation of the florigen genes (Riboni et al. 2016). It was also found that WRKY44, a member of the WRKY DNA-binding family proteins, was down-regulated by the combined activity of GI and miRNA172 (Han et al., 2013). The WRKY44 participates in sugar metabolism. Thus, the GI-miRNA172-WRKY44 may regulate drought tolerance by affecting sugar signalling in Arabidopsis (Haydon et al., 2017; Frank et al., 2018).

Mutations of GI in rice (OsGI) confer tolerance to osmotic stress created by polyethylene glycol (PEG) (Xiong et al., 2012). The osgi mutants were observed to maintain a higher water content than wild type plants by modulating stomatal closure, enhancing water utilisation and limiting transpiration leading to 'drought avoidance' (Kooyers, 2015). It is supposed that not the GI alone but the GI-CO-FT flowering time pathway controls

stomata movement (Kinoshita et al., 2011; Ando et al., 2013). It is interesting to note that *OsGI* is unaffected by osmotic stress at the transcriptional level but it is regulated at the protein level (Li et al., 2016).

Mutation of the OsGI gene in rice, activated several antioxidant genes including thioredoxin, superoxide dismutase and peroxidase making the osgi plants strong Reactive Oxygen Species (ROS) scavengers concordant with Arabidopsis, where gi mutants had increased peroxidase and superoxide levels and tolerance to paraquat and H₂O₂ (Kurepa et al., 1998; Cao et al., 2006; Li et al., 2016). Increased expression of chaperone genes in osgi leaves has been shown to improve plant tolerance to water deficits (Wang et al., 2004).

In vernalisation-sensitive *Arabidopsis* plants, exposure to cold for long duration promotes flowering via the vernalisation pathway. In contrast, a delayed flowering phenotype by the effect of FLC is observed on exposure to short-term cold or on overexpression of cold responsive genes (Seo et al., 2009; Jung et al., 2012, 2013). The *gi* mutants exhibit increased freezing tolerance along with up-regulation of cold-responsive genes. Freezing tolerance phenotype in the *gi* mutants is dependent on transcription of *CDF*. The *gi*, *cdf* double mutants are cold sensitive (Fornara et al., 2015).

Conclusions

All the above mentioned examples underline the importance of GI not only in flowering but also in the abiotic stress adaptation process. The GI genes have functions of invaluable importance and must be explored more considering their influences both directly and indirectly in the pathways. interconnected regulatory conserved functions of GI genes throw light on the possibility of their modification by genetic means in order to breed the crops that are susceptible to adverse abiotic stresses. Since GI is one of the core proteins that synchronises or indirectly impacts the level of expression of several other proteins and repressive factors that take part in plant physiological pathways, it can be concluded that GI is a strong candidate for genetic modification by modulation of its expression.

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The effects of N fertilization on soybean (*Glycine max* L. Merrill) yield and quality under different drought stress levels

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Abstract:As a result to continuous exploitation in agriculture, soil nutrients decrease, and one way of re-fertilizing is by mineral fertilization. However, applying mineral fertilizers should be controlled and pre-evaluated in terms of quantity to be added, as the excessive amounts could negatively affect both plants and soil. Fertilization is very important under abiotic stress conditions, like drought stress which has negative effects on both quantity (yield) and quality (seed content) of crops, especially drought-sensitive crops such as soybean; it is a very important legume with high content of both protein and oil.

In order to study the influence of both nitrogen fertilization and drought stress on the yield and the seed quality of two soybean cultivars, an experiment was conducted in Debrecen, Hungary in 2017. Three N fertilization rates; 0, 35 and 105 kg ha⁻¹ were applied under three irrigation regimes; severe drought (SD), moderate drought (MD) and no drought (ND). The results showed drought stress to negatively affect the yield of both cultivars by different extents; it also manipulated both protein and oil concentrations. (N) fertilization could enhance the yield of (MD) and (ND), but not (SD) treatment when applied in a relatively-low rate, whereas it negatively affected the yield when high rate was applied to (ND) treatment. The protein concentration increased as the (N) fertilization rate increased, whereas the oil concentration was not affected by (N) fertilization, but rather by drought.

It was concluded that the high-rate application of nitrogen is not always recommended for soybean, especially when water is available for plants. (N) fertilization has a noticeable effect on the protein but not on the oil concentration. Further studies on the best N rate when drought stress is applied at certain growth-stage will help to better understand the combined effects of both traits on soybean yield and quality.

Keywords: Soybean, drought stress, (N) fertilization, seed quality.

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Introduction

Soybean (*Glycine max* (L.) Merrill) has the greatest global area-harvested among seed legumes; it is the main source of relatively-cheap protein and vegetable oil (Maleki et al., 2013; Mutava et al., 2015; Wang et al., 2006). The interaction (genotype, environment) determines the ratio of protein and oil in soybean seeds (Fehr et al., 2003; Wilson, 2004). Generally, high rate of protein in soybean seeds is negatively correlated with yield (Liang et al., 2010).

Soybean yield is greatly affected by several abiotic stresses, with drought stress being one of the major ones (Fan et al., 2013); drought intensively increased over the past decades affecting the world's food security (Vurukonda et al., 2016), which makes it very important to improve the knowledge of plant response to abiotic stresses (Morison et al., 2008). Drought negatively affects quantity (yield) and quality (seed content) of soybean (Vurukonda et al., 2016) as soybean is highly-sensitive to drought

stress compared to other crops (Maleki et al., 2013) especially during certain periods of plant lifecycle (Liu et al., 2004). Many studies reported soybean seed yield, when exposed to drought stress, to be reduced (Kokubun, 2011; Li et al., 2013; Rose, 1988; Sadeghipour & Abbasi, 2012); yield reduction was found to be genotype-dependent (Bellaloui & Mengistu, 2008; He et al., 2017).

Protein and oil concentrations in soybean seeds are the most important parameters determining nutritional value (Chung et al., 2003). Under drought stress conditions, there is no effect on protein concentration (Sionit & Kramer, 1977), or less protein concentration (Boydak et al., 2002; Carrera et al., 2009; Rose, 1988; Specht et al., 2001) depending on the timing (stage) and the severity of applied drought stress (Carrera et al., 2009).

In general, protein concentration in soybean seeds is negatively correlated with oil concentration (Chung et al., 2003). Few papers reported oil concentration to be increased under drought stress (e.g. Boydak et al., 2002; Specht et al., 2001).

Nitrogen (N) is one of the most important macronutrients for plant growth and yield; it is essential for total chlorophyll content and protein synthesis. N is essentially needed for the soybean vegetative growth in order to produce optimum biomass (Fabre & Planchon, 2000; Fageria & Baligar, 2005). Biologically-fixed N₂ and mineral (N) are the two main sources of (N) needed by soybean plants (Salvagiotti et al., 2008). If there is some deficiency in fixed N₂ amounts, other sources (mainly through (N) fertilization as a quick and partially-convenient method of providing (N) to plants) must be available (Fabre & Planchon, 2000; Miransari, 2016; Yinbo et al., 1997), or else (N) from leaves will be remobilized to the seeds which, in part, will lead to decreased photosynthesis and eventually reduced yield (Salvagiotti et al., 2008). Applying (N) fertilizer at appropriate rates can enhance seedling growth by becoming established at the beginning of the season until the initiation of biological N₂-fixation by rhizobia (Ferguson et al., 2010; Seneviratne et al., 2000). Therefore, the determination of (N) fertilization influence on the growth and the yield of soybean crop is very important in order to maximize yield and economic profitability in a particular environment (Caliskan et al., 2008).

(N) fertilization is particularly very important under abiotic stress conditions (Caliskan et al., 2008) like drought stress (Obaton et al., 1982); adding (N) fertilizer to soybean increases drought tolerance as it enhances the accumulation of

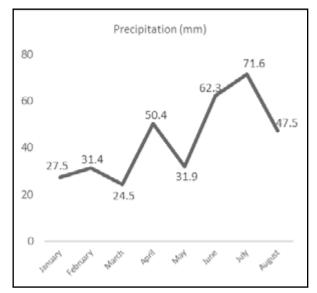
both shoot nitrogen and shoot biomass under drought stress (Purcell & King, 1996).

Our experiment aimed at revealing the effects of different (N) fertilization rates on both yield and seed quality of two soybean cultivars under drought stress conditions.

Materials and Methods

Two soybean cultivars; 'Boglár' (00 maturity group) and 'Pannonia kincse' (I maturity group) (Bonefarm, Hungary) were sown in Debrecen University's experimental site (Látókép) (N. latitude 47° 33', E. longitude 21° 27') on April 26th and harvested on September 1st, 2017. The soil type is calcareous chernozem, the average annual precipitation is 565.3 mm, whereas the precipitation between sowing and harvesting dates was 213.3 mm.

Three (N) fertilizer rates; 0, 35 and 105 kg ha⁻¹ of ammonium nitrate (NH₄NO₃) (0 N, 35 N and 105 N, respectively) were applied under three irrigation regimes; severe drought (SD) (where the precipitation amount of 213.3 mm was the only source of irrigation water), moderate drought (MD) (where an additional 50 mm of irrigation water was supplied) and no drought (ND) (where an additional 100 mm of irrigation water was supplied). The experimental design was split-split-plot design, with the cultivars being the main plots, the irrigation treatments being the sub-plots and the fertilization treatments being the sub-sub plots. The final plot number was 18 (2 cultivars



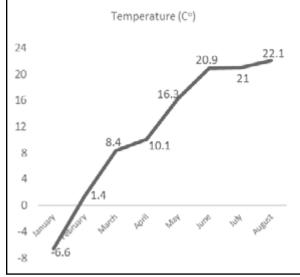


Figure 1: The precipitation (mm) and the temperature (C°) from the beginning of the year of experiment till the harvest date.

* 3 fertilization rates * 3 irrigation regimes) * 4 replications = 72 plots. The dimensions of each plot were 9.2 * 5.4 = 49.68 m² with 12 rows in each plot. Both the protein and oil concentrations were measured using NIR analyser Granolyser (Pfeuffer, Germany).

The analysis of variance (ANOVA) was conducted to compare the means of each treatment, and then tukey post-hoc test was conducted to indicate the statistically-different means using SPSS (ver.25) software.

Results and Discussion

1. Yield (kg ha⁻¹)

For cultivar 'Boglár', the fertilization rate did not play a noticeable role in the yield under severe drought stress conditions, moreover, applying (N) fertilizer insignificantly reduced the yield (to 3659 and 3753 kg ha⁻¹ for 35 N and 105 N treatments, respectively) compared to the non-fertilized control (3854 kg ha⁻¹) (table 1). Previously, Kaschuk et al. (2016) concluded that (N) fertilizer did not lead to more yield of two different soybean cultivar groups (determinate and indeterminate) whether (N) application was done at sowing time, during reproductive stages or both; same conclusion was previously reported (Hungria et al., 2006; Mendes et al., 2008). However, the fertilization did play a role in the resulted yield under moderate drought stress conditions; the yield increased as the fertilization rate increased (4576, 4717 and 4957 kg ha⁻¹ for 0 N, 35 N and 105 N, respectively) (table 1). Some researchers concluded that (N) fertilizer addition increases yield (Ham et al., 1975; Gault et al., 1984; Kuwahara et al., 1986; Nakano et al., 1987; Norhayati et al., 1988; Takahashi et al., 1991; Watanabe et al., 1986) by reducing abortions of flowers and pods (Brevedan et al., 1978). When drought was waived off, the low rate of (N) fertilizer (35 N) enhanced yield (to 5379 kg ha⁻¹), whereas, interestingly, the high rate (105 N) decreased it (to 4697 kg ha⁻¹) to a level even less than the control (0 N) (5063 kg ha⁻¹) (table 1), which implies that when plants does not suffer from stress, high rates of (N) negatively affect the yield. Fabre & Planchon (2000) reported a significant correlation between yield and (N) fertilizer during flowering stage. MacKenzie and Kirby (1979) concluded that yield was linearly correlated with (N) fertilizer amounts up to 90 kg ha⁻¹, whereas Salvagiotti et al. (2008) concluded that less than 50 kg ha⁻¹ of (N) fertilizer has lead to the largest agronomic efficiency.

The reasons for alteration in the response to (N) are not accurately specified; however, initial soil fertility, nodulation capacity, inoculant presence in soil and pre-sowing inoculation and the timing of (N) application all have a role (Gault et al., 1984; Peoples et al., 1995).

Regardless of fertilization application and rate, (SD) significantly resulted in the least yield compared to the other two irrigation regimes (table 1). It was reported that soybean seed yield decreases under drought stress conditions (Ashley & Ethridge, 1978; Bajaj et al., 2008; Dogan et al., 2007; Doss et al., 1974; Gercek et al., 2009; Heatherly & Elmore, 1986; Karam et al., 2005; Kokubun, 2011; Li et al., 2013; Rose, 1988; Sadeghipour & Abbasi, 2012; Sincik et al., 2008). The yield increased in (MD) compared to (SD), regardless of (N) fertilizer rate; this result is consistent with Dornbos & Mullen (1992) conclusion that severe drought stress reduced the seed yield of soybean more than did moderate drought stress. Moreover, the yield further increased when the drought was waived off for both (0 N) and (35 N) treatments, but decreased for (105 N) (table 1), which emphasizes the harmful effect of high (N) fertilizer rate on the expected yield.

The effect of irrigation (calculated as Eta Square) on the yield was noticeable (60.5%), which means that over 60% of the yield differences were resulted by the different irrigation regimes.

For cultivar 'Pannonia kincse', applying high rate of (N) fertilizer under severe drought stress resulted in a better yield (4276 kg ha⁻¹) compared to the low rate application (3960 kg ha⁻¹); however, the difference was not significant (table 1). This result gives an impression that (N) fertilizer could alleviate the negative effect of severe drought for this cultivar. Previous papers reported (N) fertilizer to be very important under abiotic stresses (Caliskan et al., 2008; Salvagiotti et al., 2008) such as drought stress (Lyons & Earley, 1952; Obaton et al., 1982). It was reported by Purcell & King (1996) that (N) fertilizer significantly increased the yield (to 2798 kg

Table 1: Yield (kg ha⁻¹), protein concentration (%) and oil concentration (%) of soybean cultivars 'Boglár' and 'Pannonia kincse' under different N-fertilizer rates {0 kg ha⁻¹ (0 N), 35 kg ha⁻¹ (35 N) and 105 kg ha⁻¹ (105 N)} and different irrigation regimes {severe drought (SD), moderate drought (MD) and no drought (ND)}.

	Boglár			Pannonia Kincse			
	SD	MD	ND	SD	MD	ND	
			Y	ield			
0 N	3854^{a2}	4576^{a12}	5063 ^{a1}	4335 ^{a1}	4220^{a1}	4746^{a1}	
35 N	$3659^{\mathrm{a}2}$	4717^{a1}	5379 ^{a1}	3960 ^{a1}	4325 ^{a1}	4526 ^{a1}	
105 N	3753 ^{a2}	4957 ^{a1}	$4697^{\mathrm{a}12}$	4276 ^{a1}	4185 ^{a1}	4470 ^{a1}	
			Protein Co	oncentration			
0 N	35.2 ^{a1}	34.9 ^{b1}	36.1 ^{a1}	36.1 ^{b1}	36.1^{a1}	37.8 ^{a1}	
35 N	35.1 ^{a1}	35.8^{ab1}	36.5^{a1}	36.9 ^{b1}	37.8^{a1}	38.1 ^{a1}	
105 N	36.7 ^{a1}	36.9^{a1}	36.9^{a1} 37.0^{a1}		39.2 ^{a1}	39.2 ^{a1}	
	Oil Concentration						
No N	23.5^{a1}	22.8^{a1}	$22.7^{\mathrm{a}2}$	22.7 ^{a1}	22.3 ^{a12}	21.4^{a2}	
35 N	23.4^{a1}	22.6^{a1}	22.7^{a1}	22.8 ^{a1}	21.8^{a12}	21.3^{a2}	
105 N	23.0 ^{a1}	22.6 ^{a1}	22.3 ^{a1}	22.4 ^{a1}	22.1 ^{a1}	22.2 ^{a1}	

Same number indicates no significant differences at .05 level between irrigation regimes of certain cultivar and within certain N-Fertilizer rate.

Same letter indicates no significant differences at .05 level between N-Fertilizer rates of certain cultivar and within certain irrigation regime.

ha⁻¹) compared to (2373 kg ha⁻¹) without (N) fertilizer; they related this increase to increased seed number because of decreased flower and pod abortion. Moreover, they concluded that the addition of (N) fertilizer to soybean increased drought tolerance as it enhanced the accumulation of both shoot nitrogen and shoot biomass under drought stress conditions. However, under well-watered conditions, (N) decreased yield (to 2597 kg ha⁻¹) relative to (2728 kg ha⁻¹) (Purcell and King, 1996). Chen et al. (1992) reported that under severe drought stress, every (1 kg ha⁻¹) of (N) fertilizer resulted in extra (1.2 kg ha⁻¹) seeds.

When stress was relatively moderate, the low rate of (N) Fertilizer resulted in a higher yield (4325 kg ha⁻¹) than did the high rate (4185 kg ha⁻¹) (table 1) which, similarly to 'Boglár', was the lowest; this result was also similar when the drought stress was waived off, which, once more, reflects the negative effect of high (N) fertilizer rate on the yield.

Unlike 'Boglár', the irrigation did not noticeably affect the yield of this cultivar (11.8%); Garcia et al. (2010) reported that genotypes significantly

differ in yield production under drought stress conditions and also within the interaction between drought stress and genotype; similar conclusions were reported (Bellaloui & Mengistu, 2008; Brown et al., 1985; He et al., 2017; Maleki et al., 2013). Also, the fertilization's effect on the yield of this cultivar was very low (2.2%).

2. Protein Concentration (%)

For cultivar 'Boglár' under severe drought (SD), both (0 N) and (35 N) treatments resulted in very similar protein concentrations (35.2 and 35.1%, respectively), however, reducing the severity of drought (to MD) enhanced the protein concentration for (35 N) treatment (to 35.8%), whereas decreased it for (0 N) treatment (to 34.9%). Moreover, eliminating drought stress (ND) resulted in the best protein concentration for both fertilization treatments (36.1 and 36.5% for 0 N and 35 N, respectively) (table 1). On the other hand, the high rate (105 N) resulted in the best protein concentration compared to the other (N) rates, regardless of water availability, reflecting the importance of (N) in protein

synthesis. It was previously concluded that protein content increased when (N) was increased (Ham et al., 1975); (N) fertilizer dose had a significant effect on seed protein content, as the dose of (100 kg ha⁻¹) increased seed protein just by (2%), whereas the dose of (200 kg ha⁻¹) resulted in (14%) increase in seed protein (Miransari, 2016).

In (0 N) treatment, protein concentration increased under (SD) compared to (MD), which is consistent with many papers that reported increased protein content under drought stress (Bellaloui & Mengistu, 2008; Dornbos & Mullen, 1992; Kumar et al., 2006; Rotundo & Westgate, 2009; Wang & Frei, 2011); this might be explained as a result to a reduction in seed number associated with an increase in seed size (Borras et al., 2004), or caused by remobilizing nitrogen from leaves to seeds rapidly as a result of drought stress (Brevedan & Egli, 2003; DeSouza et al., 1997) which leads to increased protein concentration.

In our experiment, protein concentration increased under (ND) treatment compared to both (SD) and (MD) treatments; few studies showed no effect (Sionit & Kramer, 1977) or lower protein concentration (Boydak et al., 2002; Carrera et al., 2009; Rose, 1988; Specht et al., 2001; Turner et al., 2005) under drought stress conditions; the relationship between drought stress and soybean seed composition remains controversial (Medic et al., 2014), and differences among the reported conclusions were suggested to be due to timing and intensity of drought stress during the different stages (Carrera et al., 2009).

The effect of (N) fertilization on protein concentration was noticeable (32.1%), whereas the irrigation effect was not (12.5%). For 'Pannonia kincse', regardless of irrigation regime, protein concentration increased as the (N) fertilizer rate increased (table 1). Rotundo & Westgate (2009) reported, in their meta-analysis study, that adding (N) fertilizer increased protein content about (27%) in all study environments; particularly, the increase was about (8%) in field studies. Increasing water availability resulted in increased protein concentration for both (0 N) and (35 N) treatments, whereas it slightly decreased it for (105 N) treatment (table 1); this tendency was different compared to 'Boglár';

Bellaloui & Mengistu (2008) suggested that the plant's response to drought stress, in terms of seed composition, might be cultivar-dependent.

Though the irrigation did not relatively affect protein concentration (3.6%), yet the fertilization noticeably did (31.8%).

3. Oil Concentration (%)

For 'Boglár', except for a slight increase in (35 N) treatment under (ND) (22.7%) compared to (MD) (22.6%), oil concentration decreased as the drought stress decreased, regardless of (N) fertilizer application and rate (table 1). Few reports showed increased oil content with water deficiency conditions (e.g. Boydak et al., 2002), whereas others indicated that water deficiency reduced oil content in the seed (Bellaloui & Mengistu, 2008; Rose, 1988; Rotundo and Westgate, 2009). The timing of drought stress was reported to have an important effect on oil content; the early-stage drought did not affect the oil content, whereas drought stress during seed filling stage resulted in a reduction of oil content by 35%. The effect of Irrigation on oil concentration was noticeable (31.6%).

Under drought stress (both SD and MD), applying (N) fertilizer decreased oil concentration; high (N) rate decreased oil concentration more than did low (N) rate, whereas when drought stress was waived off (ND), the application of low (N) rate (35 N) resulted in the same oil concentration (22.7%) as did the control (0 N); however, the high (N) rate decreased the oil concentration (to 22.3%) (table 1). The effect of fertilization was not noticeable on oil concentration (6.3%).

The correlation between oil and protein concentrations was slightly negative (r = -0.16). Chung et al. (2003) reported soybean seed protein content to negatively correlate with the amount of seed oil.

For 'Pannonia kincse', similarly to 'Boglár', decreasing drought decreased oil concentration, regardless of (N) application and rate. Under drought (whether severe or moderate), control (0 N) treatment resulted in better oil concentration compared to (105 N) treatment, whereas it was the opposite when drought was waived off (table 1). For this cultivar, the correlation between oil concentration and yield was negatively significantly-high (r = -0.44**). Same to

'Boglár', the fertilization did not relatively affect the oil concentration (1.5%), whereas the irrigation effect was noticeable (34.0%).

Conclusions

Our work was a single-year experiment only, yet some preliminary conclusions could be interpreted; it was concluded that drought stress decreases soybean yield of both studied cultivars; it also affects protein and oil concentrations to some extent. Depending on the cultivar, (N) fertilization is not always recommended for soybean, especially high rate, as it has a negative influence on the yield; however, it is important under drought stress conditions as it could alleviate the negative effect on the yield. Also, it plays an important role in increasing protein concentration in soybean seeds, whereas

it has a very little effect on the oil concentration.

More intensive research should be conducted to investigate the exact rate of (N) fertilizer under drought which leads to the best yield with maintaining relatively high protein concentration in the produced seeds. Moreover, it would be of much importance to investigate the growth stage of soybean in which nitrogen availability is mostly affected by drought stress (majorly because of N₂-fixation malfunction caused by drought), in order to apply (N) fertilizer to overcome N-deficiency negative effects.

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Investigation of the stomata size and frequency of grapevine (*Vitis vinifera* L.) cultivar 'Kékfrankos'

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Abstract: Grapevine (*Vitis vinifera* L.) leaves show high morphological diversity alongside the shoot. This variability has been investigated in this study to explore the change in leaf size, leaf thickness, stomata density and stomata size among the 1st, 5th and 10th leaves on the main shoots and leaves on the laterals. Results showed that leaf size altered from the basal abaxial leaves to the middle of the shoot, while the laterals had the smallest leaves. Number of stomata also varied significantly regarding the different levels of the canopy. First leaves on the shoots had the least stomata per unit leaf area while this number increased above. In contrast with this the size, i.e. length and width of the stomata did not differ. Leaf thickness was the lowest on the leaves of the lateral shoots, while the values decreased from the 1st to the 10th nodes. These results raised the question about the ontogeny and heteroblasty of the grapevine foliage.

Keywords: leaf morphology, canopy

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Introduction

Grapevine (Vitis vinifera L.) canopy is built up of individual leaves with variable size and diverse shape attributes. The variability is remarkable along the shoot and possibly caused by heteroblasty and ontogeny. Differences in the shape of the leaves alongside the axis were already mentioned by Ravaz (1902), although detailed explanation was given only recently (Chitwood et al., 2016). In our previous study macro-morphological variability of the canopy has been investigated. We found that basal and apical leaves on the shoots are smaller than those in the middle of the shoots, besides venation pattern and serration size are also varying (Bodor et al., 2018).

Morphology of the stomata was described in the middle of the 19th century (Anonymous, 1842). In viticulture these "pores" received more attention after the appearance of the downy mildew (*Plasmopara viticola*) in Europe, since the plant is infected throughout the stomata (Gessler et al., 2011). Stomatal openings occur most frequently on the abaxial side of the leaf. According to comprehensive studies performed in the last decade stomatal density and size of Vitis species and cultivars (Shiraishi et al., 1996), even clones (Alonso-Villaverde et al., 2011) are already known. Stomata have primary function in plant physiology, and based on previous studies their number responds to ecological circumstances (Bálo et al., 1986). Thus altitude,

row orientation (Kok and Bahar, 2015) and climatic conditions (Gokbayrak et al., 2008) can modify the stomatal density.

Although the diversity of the stomata within genotypes is well described, we have only limited knowledge about the vertical variability inside the canopy. The aim of this study was to investigate leaf size, thickness, stomatal size and distribution of 'Kékfrankos' leaves alongside the shoot (on the axis and the lateral shoot as well).

Materials and Methods

Plant material was collected during May in 2018, after berry set before *veraison*, from the experimental vineyard of the Soós István School for Oenology (Budafok, Budapest, Hungary). The experimented 'Kékfrankos' vines were trained on medium-height cordon, vertically shoot positioned. All plants were equally pruned and treated with the same canopy management.

Samples were collected randomly from several plants. Ten leaves were collected from the 1st, 5th, 10th nodes and from the lateral shoot of several shoots resulted in 40 samples altogether. Samples were digitized with a Sony A58 digital camera, and each individual leaf area was calculated with the Image J (Abramoff et al., 2004).

Two characteristics were measured on every leaf blade between the main vein and the main lateral vein: (i) Leaf thickness was investigated with a

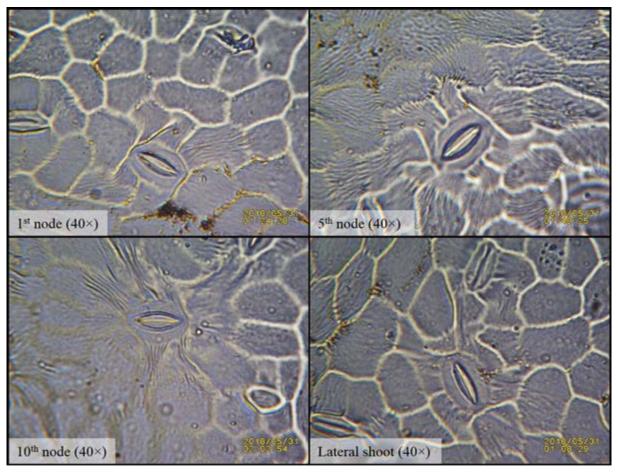


Figure 1: Stomatal imprints from the 1st, 5th, and 10th leaves of the main shoot and from the lateral shoot of the 'Kékfrankos' grapevine cultivar

digital thickness gauge (Moore and Wright Digital Thickness Gauge 053) on a 63.61 mm² surface at the same position where stomatal frequency and size were determined. (ii) Stomatal replicas were prepared with the help of a transparent nail polish collected from the lower side of all leaf samples (Figure 1). Each replica was replaced on a slide and covered with coverslip. Twenty pictures at both 10 fold and 40 fold magnification were taken from the 1st, 5th, 10th nodes and lateral shoots. For this purpose, a Bresser Digital LCD microscope was used with 5MPx resolution. Size, e.g. width and length of the stomata, was recorded with the Image J (Abramoff et al., 2004). All of the measurements were carried out twice, and correlation was calculated to detect possible errors.

Statistical analysis (mean, st. dev., rel. st. dev., correl., ANOVA) for the values of leaf area, leaf thickness, numbers of stomata, as well as stomatal width, length and shape index (width/length) was carried out with the PAST software (Hammer et al., 2002).

Results

Results are summarized in Table 1. Leaf area showed significant difference among the leaves arising from the different nodes (p<0.001). Smallest leaves were collected from the lateral shoots while the largest ones originated from the 5th nodes. Leaf thickness also showed significant (p<0.01) difference. Samples collected from the lateral shoots were the thinnest, while those originated from the nodes of the main shoot were the thickest. Numbers of stomata also proved to be significantly different (p<0.001). Lowest amount was observed on the leaves collected from the 1st nodes, while the highest was detected on the 10th node (Figure 2). Stomatal size was measured twice, and replications were statistically analysed. Linear correlation was: 0.9919 (p<0.001) which proved the accuracy of the readings. Neither width, nor length, nor stomatal shape index showed significant alteration among the different levels of the canopy.

Table 1: Morphological characteristics of the leaf samples collected from the 1st, 5th, 10th nodes and from the
lateral shoots. Superscripts indicate the significant difference (p<0.001 and p<0.01) among the samples.

Character	Position	Difference	Mean	St. dev.	Rel. st. dev.
Leaf area (cm²)	Lateral shoot		54.04a	20.89	38.67
	1st node	*	57.11a	18.38	32.19
	5 th node	•	299.47°	72.75	24.29
	10 th node		199.05 ^b	85.85	43.13
	Lateral shoot		0.26^{a}	0.02	7.26
I and think and (man)	1st node	**	0.40^{b}	0.10	24.44
Leaf thickness (mm)	5 th node		0.37^{b}	0.04	10.45
	10 th node		0.34^{b}	0.06	18.22
	Lateral shoot		117.03 ^b	26.94	32.01
Numbers of stomata/	1st node	*	94.75ª	21.67	22.87
mm^2	5 th node	•	128.82 ^b	13.99	10.85
	10 th node		156.98°	15.46	9.85
	Lateral shoot	7 . G	20.69	2.65	12.80
Stamatal width (um)	1st node		21.05	2.80	13.28
Stomatal width (μm)	5 th node	n.s.	20.98	2.77	13.20
	10 th node		19.52	2.02	10.37
	Lateral shoot		31.15	3.27	10.51
Stamatal lanath (um)	1st node		32.39	3.12	9.62
Stomatal length (µm)	5 th node	n.s.	32.05	3.93	12.25
	10 th node		30.42	2.70	8.87
	Lateral shoot		0.66	0.06	8.89
Stomatal shape index	1st node		0.65	0.05	8.08
(W/L)	5 th node	n.s.	0.66	0.07	9.90
	10 th node	0.01	0.64	0.06	9.50

^{*} significant at p<0.001, ** significant at p<0.01, n.s.: not significant

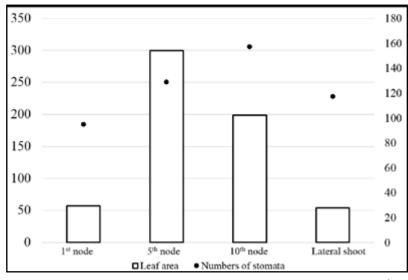


Figure 2: Leaf area and number of stomata of 'Kékfrankos' grapevine cultivar on the 1st, 5th, 10th nodes and on the lateral shoots

Discussion

Leaf area, leaf thickness and number of stomata showed significant difference among the samples collected from the 1st, 5th, 10th nodes of the main shoot and from the lateral shoots of the 'Kékfrankos' grapevine cultivar. Leaf area was 57.11 cm² on the abaxial leaves while 299.47 cm²

on the 5th node. This morphological alteration along the shoot is in accordance with the literature. Previously Demaria and Leardi (1875) have already published that leaf morphology of the grapevine is not constant, and there is a notable diversity. Thus not only the alteration of the canopy levels, but the variability within the samples collected from the same position

have importance. Relative standard deviations were calculated and these data showed that the variability of the leaf size is the lowest on the 5th node (rel. st. dev.: 24.29) and highest on the 10th node (rel. st. dev.: 43.13). Our previous study showed that leaf morphology is the most typical for a cultivar on the 9-12th nodes (Bodor et al., 2018). This is the reason why international standards also recommend leaf sampling from the middle third of several shoots, since these represent the genotype the best (OIV, 2009). The present study is in contrast with the earlier results and highlights that more cultivars in our future observations should be involved.

The values of leaf thickness were also differing among the samples, decreasing from the 1st leaf to the 10th nodes and the leaves from the lateral shoot were the thinnest. Variability was higher on the 10th node than on the 5th (rel. st. dev.: 18.22 and 10.45 respectively).

Stomatal number was the lowest on the 1st node and the highest on the 10th, with 94.75/mm² and 156.98/mm² respectively. The variability in stomatal number was the lowest at the 10th node (rel. st. dev.: 9.85), while it proved to be the highest on the samples collected from laterals (rel. st. dev.: 32.01) and from the 1st node (rel. st. dev.: 22.87). Earlier Rogiers et al. (2011) published that the position of the leaves alongside the shoot has an effect both on leaf size and stomatal density. They pointed out that leaves collected from lower nodes have less stomata than the ones higher on the shoot. These previous results are in accordance with our observations.

The difference between the size and the shape of the stomata was not significant. It suggests that this characteristic is regulated genetically while leaf position on the shoot and age of the leaf have less influence on them. On the other hand, several previous studies about the size of the stomata found significant differences among cultivars (Eris and Soylu, 1990, Boso et al., 2016), which alludes that this character is possibly not uniform. Moreover, it requires further investigations on more cultivars.

Morphological inequality among leaf samples collected from distinct nodes of the shoot can be explained with two biological reasons, namely ontogeny and heteroblasty. The first reason (*ontogeny*) explains the morphological variability with the age difference among the leaves, while the second one, i.e. *heteroblasty* (morphological) relates to the phenotypical differences of the leaves with their position on the shoot.

Regarding *ontogeny* a rather long timeframe has to be considered. New leaves arise constantly on the vine. Main leaves on the primer shoot can occur until the first trimming, while lateral shoots arise almost constantly throughout the growing season (Lőrincz and Barócsi, 2010). So the age difference of leaves can be even more than 100 days, giving significant time for ontogeny. Moreover, if phenological stages are discussed, requirements for abiotic factors and differences in ecological conditions have to be considered as well. The basal leaf is the oldest on the shoot arising at the beginning of the vegetation period, leaves in the middle of the shoot are younger, and apical leaves on the shoot top are the youngest. Beside the main shoot laterals are arising from the lateral buds. It is caused by many reasons, for example the injury/removal of the main shoot top or high vegetative performance, etc, (Kozma, 1991). The age of the leaves on the laterals is hard to defined because their formation and growing are different from the main shoots (Zufferey, 2016). This phenological difference between the oldest and youngest leaf inside the canopy can be 2 months or even more. If we consider the ecological circumstances of these phases, the alteration among the samples is not surprising. Generally, the oldest leaves (1st node) arise in April when the temperature is usually low and humidity is high, thus high evaporation is not significant. Middle leaves (5th and 10th nodes) develop days or weeks later on the same shoot when both temperature and radiation are increasing, so the environment is changing. In this study the investigated laterals had arisen approximately 1-2 weeks before sampling (middle of May). It has to be emphasised that the leaves collected from the 1st, 5th and 10th nodes were fully developed, while those collected from the laterals would increase in size, probably changing the distribution of the stomata later. As mentioned above, stomatal shape and size require further studies with more genotypes (cultivars, clones) at more phenological stages. But the obvious correlation between ecological

conditions with phenology and morphology suggests, we should complete our studies and sampling in different vineyards, wine regions, possibly in other phenological stages with more frequent "collection".

Zotz et al. (2011) concluded that *heteroblasty* has many functional reasons, such as the different light conditions, water relations or nutrient supply. In its natural circumstances grapevine is a liana like plant (Mullins et al. 2003) climbing up to the tree canopy to reach optimal light conditions. In those cases basal leaves are usually in the shade, while apical leaves reach higher radiation. In contrast with this in the vineyard cultivated plants do not have any competitors, and growers aim to provide the highest radiation to the whole canopy with minimized self-shading. In this way the initial canopy can get high radiation i.e. low self-shading in the beginning of the growing season.

Lee and Richards (1991) explained vine heteroblasty with other reasons. According to their concept (similarly to other lianas) vines have to find support during the initial phase of the growing season, consequently plants invest less source to the development of individual organs than to the apical growing in this stage.

This is in accordance with our previous (Bodor et al. 2018) and present findings: basal leaves are smaller and less differentiated than those arising from above in the middle of the shoots.

Based on the present study it can be concluded that leaf morphology and stomatal characteristics still have several unanswered questions. Further investigations are required to detect correlations of leaf morphology and stomatal characteristics with ecological conditions, phenological stages and genotypes.

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A world alimentation chance estimate based on protein production of crop species

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Abstract: Food is any substance consumed to provide nutritional support for the body. It is usually of plant or animal origin, and contains essential nutrients, such as carbohydrates, fats, proteins, vitamins and minerals. The substance is ingested by an organism and assimilated by the organism's cells in an effort to produce energy, maintain life, or stimulate growth. Food security means to provide food for anyone, recognizing the "right to an adequate standard of living, including adequate food", as well as the "fundamental right to be free from hunger".

The present paper provides information upon the results of research focusing on the protein production of some field crop species. An assessment study has been done at the Szent István University, Gödöllő to evaluate field crop species. Twelve field crop species (Sugar beet *Beta vulgaris*, spring and winter barley *Hordeum vulgare*, winter wheat *Triticum aestivum*, maize *Zea mays*, sunflower *Helianthus annuus*, peas *Pisum sativum*, potato *Solanum tuberosum*, alfalfa *Medicago sativa*, canola *Brassica napus*, rye *Secale cereale* and oats *Avena sativa*) were involved in the study.

The results obtained suggest that regarding their protein production field crop species could be sorted into three distinguished groups. Alfalfa, barley and peas were the most productive field crops and also the most economic considering the cost of protein yield. Most of the grain crops and oil seed crops formed a middle range with considerable protein formation but with highly variable costs. Spring barley was the only exception within this group since the species is dedicated basically to low protein formation patterns. The two tuber and root crops had low protein yields at high cost.

The final conclusion of the research is, that the rapidly increasing human population may have still reserves in cropland globally however crop species show some twofold differences in protein output while the price gap of that may be around 30 times wider.

Keywords: aimentation, nutrient intake, protein production

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Introduction

All living organisms rely on biochemical processes. To supply these physiological structures any of them has to have availability to certain chemical elements in the form of food. Food is any substance consumed to provide nutritional support for the living organism. It is usually of plant or animal origin, and contains essential nutrients, such as carbohydrates, fats, proteins, vitamins, minerals, fibres and water. The substance is ingested by an organism and assimilated by the organism's cells in an effort to produce energy, maintain life, or stimulate growth (WHO 2013a, 2013b). Food security means to provide food for anyone, recognizing the ,right to an adequate standard of living, including adequate food ,,, as well as the "fundamental right to be free from hunger, (WHO 2004, Lean 2015).

The human population of the world has been increasing in an unprecedented pattern during the past century. By the time of our study the global population has reached 7.7 billion and it is expected to be over 11 billion (Figure 1.) by the end of the 21st Century. The problem of this increment is twofold. On one hand this enormous amount of human beings will have to be supplied with adequate quantity and quality of food. The other is the environmental impact of this demographic phenomenon, namely the environmental footprint of the human society (Várallyay 2008). Frankly it has to be identified how much is the influence of ours and how can we control this impact.

Crop site × crop plant interactions have a profound role in yield formation regarding crop yield quantity and quality (Pepó 2010,

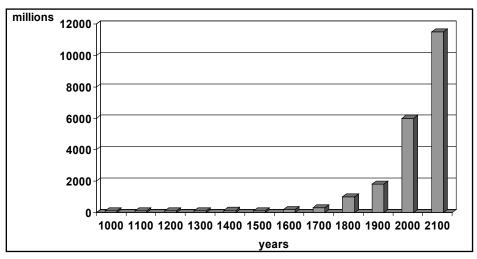


Figure 1: World population increment. Source: Montgomery 2014

Tarnawa et al. 2006, 2011 and 2018). Plant development depends on optimum environmental conditions from among which water availability, nutrient supply and photosynthetic processes may influence yield formation and the manifestation of quality characteristics.

Climate change research results in Hungary have highlighted the variation induced by water availability on protein formation of field crops (Kassai et al. 2019; Eser et al, 2019; Jolánkai et al 2018, 2019).

The alimentation of the human race is based on agricultural activities including field crop production and its output converted into food and feed. All biochemical processes that are yielding the essential groups of food are supplied by a unique process, the only active carbon sequestration; the photosynthesis. The highest amount of converted chemical compounds containing C, H, O and N are the carbohydrate substances including monosaccharides and polysaccharides as well as triglycerides forming various fats, and last but not least proteins which are built from a wide range of amino acids, many of them having no abilities to be produced by certain living organisms, therefore such compounds are to be obtained from other live individuals within the food chain.

Field crops represent therefore a sort of a basis of almost all food and feed products and in an indirect way of higher levels of animal food conversion that are intended to be consumed by humans. Field crops have a high variation regarding their botany, agronomic patterns, and

the yield and its chemical properties (Hohls 1995, Jolánkai et al 2018, Kassai 1994, Máté et al 1993). In this study the twelve most widespread field crop species have been studied including grain crops, oil seed crops, root and tuber crops and leguminous forages. From among the nutritional compounds protein output of field crop species was evaluated since these chemical structures provide a common basis for comparison between plant yields.

Materials and methods

The materials and methods of the present study cover a rather broad field, since there are various topics of research work done by the SIU Crop Production Institute, Hungary. Majority of the results are based on experimental research, however, some evaluations were implemented by using national public data, or observation results published (FM 2017, FAOSTAT 2017).

An assessment study has been done by the authors to evaluate and identify the agronomic parameters of protein yield of field crop species. Twelve field crop species (Sugar beet Beta vulgaris, spring and winter barley Hordeum vulgare, winter wheat Triticum aestivum, maize Zea mays, sunflower Helianthus annuus, peas Pisum sativum, potato Solanum tuberosum, alfalfa Medicago sativa, canola Brassica napus, rye Secale cereale and oats Avena sativa) were involved in the study. Evapotranspiration patterns (ET) of the crops studied have been identified and physiologically reliable protein ranges within crop yields were evaluated.

Table 1: Protein production	of twelve crop plant species. SIU, 2017
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Crop	protein %	crop yield t ha-1	protein yield kg ha ⁻¹	Crop price 1000 HUF t ⁻¹	Cost of 1 kg protein output HUF	DRI/ha person
Medicago sativa	18.0	4.35*	783	22.5	125.0	42.9
Solanum tuberosum	2.0	24.9	498	65.7	3285.0	27.2
Beta vulgaris	1.1	41.2	453	15.0	1364.2	24.8
Triticum aestivum	13.0	4.8	624	45.0	346.1	34.1
Hordeum vulgare	16.5	4.1	676.5	43.5	263.6	37.0
Hordeum vulgare distichon	11.2	3.7	414.4	45.0	401.8	22.6
Secale cereale	12.8	4.2	537.6	43.8	342.1	29.4
Avena sativa	13.6	4.4	598.4	44.1	324.2	32.7
Zea mays	9.5	5.8	551	46.9	493.7	30.1
Helianthus annuus	18.5	3.3	610.5	93.0	502.7	33.4
Brassica napus	22.6	3.2	723.2	105.0	500.0	39.6
Pisum sativum	24.0	2.8	672.0	71.0	295.8	36.8

^{*}hay

In the study experimental mean values of identical agronomic treatments and homogenized bulk yield samples were used only. Precipitation records have been evaluated in relation with yield quantity and quality. Quality characteristics were determined at the Research Laboratory of the SIU Crop Production Institute, according to Hungarian standards (MSZ, 1998, Győri 2006, Győri 2008). Analyses were done by statistical programmes with respect to the methodology of phenotypic crop adaptation (Eberhart and Russell 1966; Finlay and Wilkinson 1963; Hohls, 1995). The meteorological database of the research referring to precipitation as well as temperature data was provided by the Hungarian Meteorological Service (OMSZ). Statistical evaluations, crop ecological model adaptations, and calculations were done by regular methods (Sváb, 1981; Finlay and Wilkinson, 1963).

The alimentary evaluations of the field crop species studied were done in accordance with WHO (2004, 2013a, 2013b), Lean (2015) and Eser et al (2019). Dietary Reference Intake (DRI) estimates were applied by methods in accordance with Gunnars (2018).

The present paper produces results of an ongoing research in relation with weather impacts on quality and quantity of crop production (Kassai et al. 2019, Tarnawa et al. 2006, 2011 and 2018, Jolánkai et al. 2018, 2019, Eser et al 2019).

Such an assessment has a diverse nature. Once, it is beneficial regarding the abundance and the duration of baseline data. On the other hand, it is restricted to the available structure and moreover it is bound mainly to available figures giving less chance for deep layer evaluations. However, the study could provide some novel specific information on crop performance in relation with food security.

Results and discussion

The results obtained suggest that regarding their protein production, field crop species could be sorted into three distinguished groups. Alfalfa, barley and peas were the most productive field crops and also the most economic considering the cost of protein yield. Most of the grain crops and oil seed crops formed a middle range with considerable protein formation but with highly variable costs. Spring barley was the only exception within this group since the species is dedicated basically to low protein formation patterns. The two tuber and root crops had low protein yields at high cost (Table 1.).

The final conclusion of the research is that the rapidly increasing human population may have still reserves in cropland globally however crop species show some twofold differences in protein output while the price gap of that may

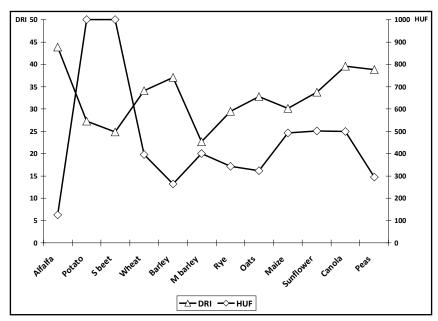


Figure 2: DRI supply and the cost of protein of twelve crop plant species. SIU, 2017

be around 30 times wider (Figure 2.). Today there is no alternative to field crop production, and so no other major resources for human alimentation than agriculture. From the Neolithic age mankind had to move from hunting and gathering to agricultural activities providing food and feed rather than exploiting natural ecosystems. Nowadays with an exception of ocean fisheries, most of the alimentation of mankind is based on human controlled inputoutput systems using photosynthetic energy conversion. Concerning Dietary Reference Intake demand one hectare of land may supply

20 to 40 average adults according to the species produced. This theoretic value was based on experimental conditions therefore commercial production can be much different from that. More research is needed to precise crop plant × crop site interactions with a special view on global spatial performance.

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OBITUARY

György VÁRALLYAY (1935 - 2018)

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It is not easy to accept losses. The most crucial losses in our life we ever experience is the death of a person who had profound influence in making and managing a better society. Professor Várallyay was founder and a most active editorial board member of Columella Journal of Agricultural and Environmental Sciences. On one hand he was a scientist, an excellent pedologist, an appreciated and renowned professor. On the other hand he was much more than that. He was a wise man with an ability to access anyone, and that made him to be a most successful person disseminating knowledge and teaching the public.



György VÁRALLYAY (1935 - 2018)

He was born in an academic family on the 17th of July 1935 in Debrecen. His father himself was a professor engaged in soil science at the famous Mosonmagyaróvár College. We may assume that György Várallyay has been initiated to follow the family traditions from his childhood.

He was student of the Gödöllő Agricultural University, wherefrom he graduated in 1957. From the very beginning he was involved in soil research. Between 1957 and 1960 he was employed by the National Institute of Agricultural Quality Testing (OMMI) where he was a junior research fellow experiencing in soil mapping, melioration and extension.

In 1960 he was appointed by the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences (RISSAC), his second and last workplace. He has been involved in various research programmes aiming the exploration, remediation and utilization of saline soils which represent a huge tract within arable lands in Hungary. This research have yielded his first scientific degrees; a PhD in 1964 and a CSc in 1968. During these years he started his active participation in the international scientific community. He became a member of the International Soil Science Society, and also, he joined an expert mission in Jemen.

1969 can be considered as the beginning of a new phase in his scientific research. After completing a scholarship in the Netherlands, he introduced novel methodology in the field of soil physics and water management in Hungary. His scientific research results contributed to almost all methodology standards in these fields.

From 1976 he was the head of the Soil Science Department of RISSAC. He was a key member of the nationwide research programme "The determination of the agro-ecological potential of Hungary" lead by István Láng. During this work he managed to design a series of 1:100.000 scale soil maps of Hungary. Between 1981 and 1997 he was the director of the institute. According to his high skills in management and coordination, he became one of the most successful leaders within the scientific network of the Hungarian Academy of Sciences.

The scientific activities, his contribution to the national and international scientific organisations is enormous. He has been a member, secretary and later president of the Soil Science Committee of the Hungarian Academy of Sciences. He has also been an active participant of high level governmental bodies in the field of scientific qualification and environmental decision making. He has been a member of the highest scientific committees of all the four Hungarian agricultural university faculties. He defended his DSc thesis in 1988. He was elected to be the member of the Hungarian Academy of Sciences (1993 CM; 1998 FM). He was awarded to be an external member of the Slovak Academy of Sciences. He was a founder of the Alps Adria Scientific Cooperation, an organisation integrating scientists of various countries of the geographic region. He was the president of the Hungarian Soil Science Society between 1990 and 1999.

His exceptional scientific output is labelled with more than 800 scientific publications and almost 2000 citations referring to those. He was editorial board member of a wide range of scientific journals (Acta Agronomica Hungarica, Archives of Agronomy and Soil Science, Columella, Geoderma, Hidrológiai Közlöny, International Agrophysics, Land Degradation and Rehabiliation, Soil Technology). Also he was the editor-in-chief of the Agrokémia és Talajtan from 1997 to 2014.

He was appreciated by many national and international scientific awards. Some of the most important ones: Magyar Köztársasági Érdemrend Középkeresztje 1997, Széchenyi Prize (2004), and last but not least the highest award in the field of crop production - the Surányi insignum (2015).

Professor György Várallyay left us. We miss him. He was a good friend, a wise man, a learned scientist, a brilliant teacher and last but not least an active member of our community. Simply we may state that he was a man of spiritual power, with a mission to enrich society.

Source of the graphics

Front cover:

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

Rear cover:

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



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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^{th} Century.