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REVIEW PAPER

Crop breeding for a changing climate in the Pannonian region: towards integration of modern phenotyping tools

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Abstract

The Pannonian Plain, as the most productive region of Southeast Europe, has a long tradition of agronomic production as well as agronomic research and plant breeding. Many research institutions from the agri-food sector of this region have a significant impact on agriculture. Their well-developed and fruitful breeding programmes resulted in productive crop varieties highly adapted to the specific regional environmental conditions. Rapid climatic changes that occurred during the last decades led to even more investigations of complex interactions between plants and their environments and the creation of climate-smart and resilient crops. Plant phenotyping is an essential part of botanical, biological, agronomic, physiological, biochemical, genetic, and other omics approaches. Phenotyping tools and applied methods differ among these disciplines, but all of them are used to evaluate and measure complex traits related to growth, yield, quality, and adaptation to different environmental stresses (biotic and abiotic). During almost a century-long period of plant breeding in the Pannonian region, plant phenotyping methods have changed, from simple measurements in the field to modern plant phenotyping and high-throughput non-invasive and digital technologies. In this review, we present a short historical background and the most recent developments in the field of plant phenotyping, as well as the results accomplished so far in Croatia, Hungary, and Serbia. Current status and perspectives for further simultaneous regional development and modernization of plant phenotyping are also discussed.

Keywords: Agriculture, climate change, Pannonian region, plant breeding, plant phenotyping.

Introduction

Major agricultural crops in the Pannonian region

Pannonian countries with the largest areas of their territories extending into the Pannonian Plain are Hungary, Serbia, Croatia, and Romania. In these areas, the agroecological conditions are suitable for the cultivation of field crops, with cereal production being the most important and accounting for 60–65% of total arable land, followed by industrial crops at 12–18% [Hungarian Central Statistical Office (http://www.ksh.hu/?lang=en%20htm; National Institute of Statistics of Romania (https://insse.ro/cms/en); Statistical Office of the Republic of Serbia (https://publikacije.stat.gov.rs/G2020/PdfE/G20202053.pdf; The Republic of Croatia—Central Bureau of Statistics (https://www.dzs.hr/default_e.htm)].

Maize, wheat, and barley are the most important cereal crops in the Pannonian region, contributing 30, 10, and 6% to European production, respectively (FAOSTAT https://www.fao.org/faostat/en/#data). Based on the harvested areas in the Pannonian region, maize ranks first with 2.2 Mha, >40% of which is in Hungary. The average yields during 2017–2019 varied from 7.3 t ha^{-1} in northern Serbia to 8.1 t ha^{-1} in northern Croatia and western Romania (Table 1). Wheat and barley are the two most grown winter cereals in the region for food and feed, with a 3 year average harvested area of >1.8 Mha and nearly 395 000 ha, respectively. The average yields range from 4.7 t ha^{-1} to 5.6 t ha^{-1} for wheat and from 4.2 t ha^{-1} to 5.2 t ha^{-1} for barley (Table 1). Frequent drought periods are a considerable threat to cereal crop yield stability and, in maize, they can cause significant yield losses, ranging from 20% in moderately dry years (Pandžić *et al.*, 2021, Preprint) to >60% during a severe drought (Popova and Kercheva, 2005; Stricevic *et al.*, 2011).

The most important oil crops in the Pannonian region are sunflower, soybean, and rapeseed, used for human consumption, animal feed, biodiesel, and pharmaceutical production. The region contributes 18, 16, and 7% to the total European sunflower, soybean, and rapeseed production, respectively (FAOSTAT https://www.fao.org/faostat/en/#data). In the four Pannonian areas, sunflower is cultivated on >1 Mha, with an average yield of 3 t ha^{-1} (Table 1). Hungary is the largest producer of sunflowers among the Pannonian countries, accounting for almost 60% of their production. In the Pannonian parts of Croatia, Romania, Serbia, and Hungary, soybean is produced on >392 000 ha and its average yields ranged from 2.4 t ha^{-} to 2.9 t ha^{-1} (Table 1). Serbia, with >203 000 ha cultivated under soybean in its Pannonian part, is one of the leading European producers and the largest in the region. In the four largest Pannonian countries, rapeseed is grown on nearly 467 000 ha, with two-thirds of this area in Hungary (Table 1). The average yield varied among the countries from 2.6 t ha^{-} to 3 t ha^{-1}. While changes in climate are expected to have an adverse effect on yield of most crop species, in soybean and other C_3 crops climate change is expected to have positive effects due to higher concentrations of carbon dioxide. These changes will also lead to an increased need for irrigation for almost all crops (Jancic *et al.*, 2015). In sunflower, simulations of predicted climate changes showed the spread of growing area and an increase of sunflower yield for northern parts of Europe, with the potential occurrence of negative effects on yield in southern latitudes (Debaeke *et al.*, 2017). The same holds for rapeseed, where the effects of climate change for a no-adaptation scenario are predicted to be more favourable for rapeseed production in cooler regions, causing a shift of rapeseed-growing zones from southern to northern Europe (Donatelli *et al.*, 2015).

Table 1. The acreage, production, and average yield of the main agricultural crops in different regions of the Pannonian Basin averaged for the period 2017–2019[a]

Crop	Area (ha)	Production (t)	Yield (t ha^{-1})
Hungary			
Wheat	1 002 730	5 294 133	5.3
Maize	985 164	7 683 145	7.8
Sunflower	625 201	1 853 154	3.0
Rapeseed	311 388	948 989	3.0
Barley	253 207	1 315 059	5.2
Soybean	65 339	176 697	2.7
Northern Serbia			
Maize	585 615	4 223 704	7.3
Wheat	341 299	1 680 299	4.9
Sunflower	204 929	619 037	3.0
Soybean	203 665	571 212	2.9
Barley	48 749	224 245	4.6
Rapeseed	28 296	82 393	2.9
Northern Croatia			
Maize	244 697	1 994 059	8.1
Wheat	129 532	730 361	5.6
Soybean	80 146	232 329	2.9
Barley	49 932	244 500	4.9
Rapeseed	48 336	131 851	2.7
Sunflower	36 704	110 939	3.0
Western Romania			
Maize	453 278	3 668 323	8.1
Wheat	338 718	1 607 514	4.7
Sunflower	184 385	558 433	3.0
Rapeseed	80 900	214 001	2.6
Soybean	43 426	105 526	2.4
Barley	43 001	181 066	4.2

[a] Hungarian Central Statistical Office (http://www.ksh.hu/?lang=en%20htm; National Institute of Statistics of Romania (https://insse.ro/cms/en); Statistical Office of the Republic of Serbia (https://publikacije.stat.gov.rs/G2020/PdfE/G20202053.pdf; and The Republic of Croatia—Central Bureau of Statistics (https://www.dzs.hr/default_e.htm).

Agro-ecological conditions of the Pannonian region in the context of climate change

The Pannonian region is a notable geomorphological and ecological entity of Europe, with agriculture playing an important role in the region's economy. It is a large flat basin that is situated in the south-eastern part of Central Europe (Fig. 1). This region encompasses several countries, namely small parts of north-east Austria and Slovenia, the south-east of the Czech Republic, the southern lands of Slovakia, the whole of Hungary, northern areas of Croatia, small parts of northern Bosnia and Herzegovina, north Serbia, western Romania, and south-western Ukraine. Enclosed by the Alpine, the Carpathian, and the Dinaric mountain ranges and intersected by the Danube and Tisa rivers, its flat area features distinctive biodiversity and agroclimatic conditions (European Environment Agency, 2002). The climate in the Pannonian Basin is continental, with considerable annual temperature variations from hot summers to cold winters, including both hot-summer humid continental and warm-summer humid continental climate types (Peel *et al.*, 2007; Beck *et al.*, 2018). The review will particularly focus on the more homogenous central and the southern parts of the Pannonian region, which are characterized by a continual alluvial plain, large grassland ecosystems, fertile humus-rich chernozem soils, and climate type with hot summers.

In recent decades, the negative impact of climate change on agricultural production has been observed in the Pannonian region, reflected in erratic rain patterns with unfavourable distribution, frequent events of extreme droughts, dry winds, heat waves, hailstorms, and disease outbreaks (Punge and Kunz, 2016; Ceglar *et al.*, 2018; Crocetti *et al.*, 2020; Jevtić *et al.*, 2020). Among the European environmental zones, the Pannonian basin had more pronounced warming and decreased precipitations between April and September, increased variability of autumn sowing conditions, etc. All this together could considerably hinder potential crop productivity under rain-fed conditions (Trnka *et al.*, 2011). Model-based climate change projections marked the Pannonian Basin, rather than the Mediterranean, as the most negatively affected European area in terms of changing climate, with a profound increase in drought duration, intensity, and variability by 2050 (Olesen *et al.*, 2011; Trnka *et al.*, 2011). Such current and predicted extreme weather conditions present a serious threat to rain-fed field crop production.

The increased accumulation of active temperatures necessary for the development of specific phenological phases for each crop, also known as growing degree-days, contributed both to lengthening of the crop growing season and to extending the crop growing range to northern latitudes (Peltonen-Sainio *et al.*, 2009). Considerable temperature variability across the Pannonian Plain imposes a serious risk of crop damage from low winter temperatures in the absence of snow cover and late spring frosts, with spring and/or early summer heat waves additionally decreasing crop productivity (Elsgaard *et al.*, 2012; Eitzinger *et al.*, 2013). Besides direct effects on the crops, the climate change in the Pannonian Basin also strongly affect complex inter-related processes in the soil and its properties. They influence water-holding capacity and availability, microbial activity and mineralization of soil organic matter, salinity, erosion, desertification, carbon and nitrogen flows, and nutrient content and availability (Baranka *et al.*, 2017).

Plant phenotyping technologies: achievements and challenges

In traditional breeding, phenotyping is based on visual assessment or measuring numerous plant traits, requiring a lot of

Fig. 1. Topographic map of the Pannonian Basin created at https://apps.nationalmap.gov/viewer/.

time and intensive labour. Besides that, this type of assessment precludes tracking of the plant phenotype at physiological and biochemical levels (Li *et al.*, 2021). Modern plant phenotyping is based on non-destructive measurements of a trait over time and high-throughput measurements, to screen many genotypes under similar conditions (Costa *et al.*, 2019a). Significant progress has been made over the last decade in image-based phenotyping, starting from the characterization of single plant traits in controlled conditions to high-throughput field techniques in plant plots and canopies capturing phenotyping data in time and space (Fritsche-Neto and Borém, 2015; Walter *et al.*, 2015). High-throughput plant phenotyping platforms (HTPPs) in controllable environments are based on: read green blue (RGB) imaging, chlorophyll fluorescence imaging, hyperspectral imaging, thermal imaging, and light detection and ranging (LiDAR) (Zhao *et al.*, 2019). Currently, the most common field-based phenotyping platforms (FBPPs) include satellite imaging, unmanned aerial vehicles (UAVs), and proximal phenotyping using ground-based vehicles, cameras, and sensors (Chawade *et al.*, 2019).

A detailed overview of the HTPPs used under environmentally controlled and uncontrolled conditions was provided by Li *et al.* (2021). Platforms were used for tracking numerous traits such as canopy height and temperature, crop lodging, radiation dosage, drought and nitrogen stress, disease severity, efficiency of water use, crop growth rate, normalized difference vegetation index(NDVI), photosynthesis, etc. Besides significant and fast progress, phenotyping requires further development assisted by experts in the field of plant and biological sciences including—omics, hardware, software and sensor development, and bioinformatics (Costa *et al.*, 2019a;Yang *et al.*, 2021). These advances are expected to further improve the efficacy of plant selection and breeding and to reduce inevitable crop losses during production by monitoring plant health and responses to environmental challenges (Chawade *et al.*, 2019).

Understanding crop/plant responses to the environment is crucial to generate genotypes for different environmental conditions and to achieve crop stability and plasticity. In this context, the ability to integrate information obtained by phenotyping across different levels of biological organization (molecules, cells, tissues, organs, whole plants, and canopies) is also very important. The lack of such integrated data is a general drawback in plant phenotyping. There are three major phenotyping descriptors: throughput (number of units processed per unit time), resolution (distance between two spatial or time points), and dimensionality (number of captured traits). According to Dhondt *et al.* (2013), in practice, a trade-off between these descriptors usually occurs due to economic, technological, time, and personnel constraints, causing difficulties in this type of data integration. Thus, phenotyping at organ and cellular levels is undertaken at low throughput but (potentially) high dimensionality, while, in contrast, whole-plant phenotyping is operated on a high-throughput but low dimensionality basis. To overcome this gap, the increased dimensionality in high-throughput plant phenotyping alongside increased throughput in molecular and cellular phenotyping is needed (Costa *et al.*, 2019a). Moreover, integration of phenotyping data with increased knowledge of crop physiology and data obtained by different omics technologies is very important for enhanced phenotyping of crops and accurate description of complex agronomically important traits, such as yield, quality, and responses to biotic and abiotic stresses.

Large sets of complex data generated by plant phenotyping are not only valuable for monitoring crop conditions, changes in nutrients availability, disease occurrence, etc., but can also be used for modelling and meta-analyses. New advanced bioinformatics tools and machine learning models (MLMs) for data integration are useful for different tasks. Non-linear models, such as random forest regression (RFR) and neural networks, have certain advantages over linear models mostly because in living systems relationships between variables are non-linear. Apart from regression analysis, statistical models that use artificial neural network (ANN) models are capable of establishing patterns and correlations among data (Van Klompenburg *et al.*, 2020) and can be used for yield predictions (Niedbała *et al.*, 2019; Rajković *et al.*, 2022). Also, it is essential that the datasets are findable, accessible, interoperable, and reusable (FAIR standard) in such a way that they can be analysed by different users. This requires software interfaces providing different levels of access to different users to allow data analysis in relation to environmental conditions. In addition, data organization and storage need to be done in a secure way over long periods; the data can be interpreted in a biological context and used for meta-analyses of experiments.

Initiatives for development and networking of phenotyping infrastructures

The EMPHASIS project (https://emphasis.plant-phenotyping.eu) was set up under the international mechanism of the European Strategy Forum for Research Infrastructure (ESFRI, http://www.esfri.eu) with the aim of establishing and integrating the European plant phenotyping infrastructure, enabling researchers to use facilities and resources for plant phenotyping under diverse environmental conditions across Europe, and promoting future food security in a changing climate. The first steps toward this initiative in Europe were made by three projects: the COST Action FA1306 (http://www.cost.eu/COST_Actions/fa/FA1306), the European Plant Phenotyping Network (EPPN; http://www.plant-phenotyping-network.eu/), and EPPN2020 (https://eppn2020.plant-phenotyping.eu/). On a global level, the International Plant Phenotyping Network (IPPN) was established in 2016 with the aim of promoting plant phenotyping research by establishing a global institutions network, fostering communication of stakeholders, increasing plant phenotyping visibility and impact, and improving research through interdisciplinary training (https://www.plant-phenotyping.org). Based on the surveys of the current status of global phenotyping by IPPN, the phenotyping infrastructures at the global level are mostly concentrated in Europe and Australia (Yang *et al.*, 2020), where national research infrastructure platforms are gathered in the Australian Plant Phenomics Facility (APPF). An increase in the investment in plant phenotyping facilities was recorded in the USA and Canada, resulting in the establishment of their regional plant phenotyping network (North American Plant Phenotyping Network—NaPPN; https://www.plantphenotyping.org/). Similar initiatives are also present in Latin America (Latin American Plant Phenomics Network; LatPPN) and China (China Plant Phenotyping Network; CPPN). The same authors stated, after searching crop phenomics or high-throughput crop phenotyping-related papers in the Web of Science, that the most common crops were wheat (30%), maize (17%), rice and Arabidopsis (each one 13%), barley (7%), and other crops (20%), while the number of published papers related to crop phenomics has significantly increased in the past 20 years.

It is hard to evaluate the position of the Pannonian region in the global and European plant phenotyping community without considering large differences among the countries at the level of development and application of plant phenotyping technology. The Central European countries such as Austria, Czech Republic, Slovakia, and Hungary have a higher level of overall technological development and, as a consequence, a higher level of infrastructure development for plant phenotyping. For example, the Austrian Plant Phenotyping Network (APPN) was established in 2017 by the representatives of the University of Natural Resources and Life Sciences, University of Vienna, University of Innsbruck, and Vienna BioCenter Core Facilities. The Plant Science Facility of the Vienna BioCenter was the main initiator of the APPN and presently provides state-of-the-art equipment and expertise for environmental simulation, multisensor high-throughput plant phenotyping of small and large plants, and subsequent image and data analysis (https://www.viennabiocenter.org/vbcf/plant-sciences/). Likewise, Photon Systems Instruments (PSI), a global producer of automated phenotyping technologies located near Brno, played a major role in placing the Czech Republic on the European and global plant phenotyping map. Consequently, PSI, Masaryk University, and Palacký University signed a memorandum of creation of the Czech Plant Phenotyping Network (CzPPN) in order to increase competitiveness and offer the technology and expertise to other parties, and demonstrate successful cooperation of academia and industry. In neighbouring Slovakia, the Slovak University of Agriculture (SUA) in Nitra with its Slovak PlantScreen™ Phenotyping Unit (SPPU) emerged as a leading national plant phenotyping institution. Although the national phenotyping network has not yet been established, SUA with SPPU supported both EMPHASIS and EPPN2020 infrastructure projects and significantly contributed to the plant phenotyping research community in Europe. A similar situation occurred in Hungary where plant phenotyping research was established 15 years ago based on the initiative of the Biological Research Centre in Szeged with the contribution of Cereal Research Ltd, Szeged, and later supported by the EPPN and EPPN2020 infrastructure projects and the COST Action FA1306 initiative. In contrast to the above, countries such as Slovenia, Bosnia and Herzegovina, and Romania are still at the point of establishing national plant phenotyping initiatives, without any noticeable phenotyping infrastructure, but potentially finding their opportunities in transnational access to phenotyping installations through EPPN2020 (https://eppn2020.plant-phenotyping.eu/Selected_Projects). The actual state and experiences of crop phenotyping in Hungary, Croatia, and Serbia, the largest production areas in the Pannonian Plain with similar agro-ecological conditions and intertwined historical and cultural backgrounds, will be further elaborated in this review.

Achievements in climate resilience improvement using breeding tools

Exploiting diversity of crop genetic resources in the Pannonian region

Crop genetic resources in the Pannonian countries encompass traditional varieties, local landraces, genetic stocks, breeding material, synthetic populations, and crop wild relatives. They are stored and managed *ex situ* in gene banks and research institute collections across the region, and in some cases maintained on-farm, while less often some of them can be found *in situ* in their natural habitats. According to the Genesys Global Portal on Plant Genetic Resources (https://www.genesys-pgr.org), >22 360 maize, 35 300 wheat, 17 029 barley, 4600 soybean, 1300 sunflower, and 1000 rapeseed accessions of the main field crops genetic resources are kept *ex situ* in 22 gene banks and institutes in the Pannonian countries (Table 2). The number of accessions of these six main field crops is probably much higher, considering that the collections from different countries are unequally represented in the database, due to the unavailability of data on crop genetic resources from some institutions (Anđelković *et al.*, 2020). However, in the Pannonian region, crop genetic resources are largely unexplored sources of potentially important genes for climate resilience improvement, since local breeders are mainly interested in working with elite breeding material, while other less advanced germplasm, with few exceptions, has been neglected in breeding programmes of major crops.

To more efficiently utilize a huge number of accessions in gene banks for the introduction of traits related to climate resilience, the concept of core collections based on the selection of a small manageable subset that retains maximum diversity of the whole collection was successfully used for germplasm characterization and evaluation in the leading breeding centres of the

Table 2. Plant genetic resources for food and agriculture of the main field crops in the genebanks of the Pannonian countries and worldwide genebanks according to the Genesys Global Portal on Plant Genetic Resources

Crop	Country	Country accessions[a]	Country holding institutes[b]	Accessions worldwide[c]	Holding institutes worldwide[d]	Holding countries worldwide[e]
Maize	Austria	62	3	127	12	9
	Bosnia and Herzegovina	11	1	343	4	4
	Croatia	252	4	675	11	8
	Czech	791	1	86	6	5
	Hungary	2919	1	1796	17	15
	Romania	6756	5	6607	19	12
	Serbia	5475	1	53	2	2
	Slovakia	747	1	239	10	8
	Slovenia	NA	NA	115	4	4
	Ukraine	9014	3	7303	12	10
Wheat	Austria	1279	3	2860	26	20
	Bosnia and Herzegovina	136	1	219	6	6
	Croatia	146	4	582	20	15
	Czech	11 072	1	1633	23	20
	Hungary	7830	1	4216	31	25
	Romania	2791	5	2817	26	20
	Serbia	NA	NA	1576	8	8
	Slovakia	4006	1	1156	15	13
	Slovenia	NA	NA	23	8	8
	Ukraine	8084	2	5034	31	23
Sunflower	Austria	NA	NA	8	5	5
	Croatia	NA	NA	3	1	1
	Czech	93	1	8	2	2
	Hungary	31	1	632	7	7
	Romania	185	2	346	10	8
	Serbia	NA	NA	5	2	2
	Slovenia	NA	NA	3	2	2
	Ukraine	1019	2	745	6	5
Barley	Austria	889	3	1693	25	20
	Bosnia and Herzegovina	34	2	127	5	5
	Croatia	97	2	224	13	12
	Czech	5224	1	1661	28	21
	Hungary	4398	1	1475	27	20
	Romania	3498	4	1059	24	17
	Serbia	NA	NA	281	13	10
	Slovakia	1716	1	513	12	10
	Slovenia	NA	NA	74	7	7
	Ukraine	4671	2	2704	21	17
Soybean	Austria	41	1	22	10	9
	Bosnia and Herzegovina	NA	NA	2	1	1
	Croatia	28	1	22	3	3
	Czech	247	1	94	7	5
	Hungary	756	1	331	9	11
	Romania	1060	3	344	11	9
	Serbia	NA	NA	25	2	2
	Slovakia	513	1	63	3	3
	Slovenia	2	1	4	2	2
	Ukraine	2050	2	867	11	9

Table 2. Continued

Crop	Country	Country accessions[a]	Country holding institutes[b]	Accessions worldwide[c]	Holding institutes worldwide[d]	Holding countries worldwide[e]
Rapeseed	Austria	20	3	21	9	6
	Croatia	15	1	NA	NA	NA
	Czech	824	1	50	3	3
	Hungary	73	1	32	3	3
	Romania	81	2	61	7	5
	Serbia	NA	NA	1	1	1
	Ukraine	2	1	212	11	8

[a] Number of accessions in each country;
[b] Number of the institutes from the country that maintain the crop accessions.
[c] Number of the crop accessions originating from the given country that are held in other parts of the world.
[d] Number of institutes worldwide that hold the crop accessions originating from the given country.
[e] Number of other countries worldwide that hold the crop accessions originating from the given country
NA, data not available.

Pannonian countries. Phenotyping and/or genotyping of core collections enabled identification of genotypes with desirable traits, such as drought tolerance (Mikić *et al.*, 2016) and grain quality in maize (Ignjatović-Micić *et al.*, 2014; Vančetović *et al.*, 2015), earliness, yield, and yield-related traits in wheat (Börner *et al.*, 2011; Dodig et al., 2010a, 2012a; Brbaklić *et al.*, 2015; Trkulja *et al.*, 2019) and in barley (Brbaklić *et al.*, 2021); maturing time, yield, and protein and oil contents in soybean (Miladinović *et al.*, 2018a; Haupt and Schmid, 2020); mineral content in common bean (*Phaseolus vulgaris* L.) (Gunjača *et al.*, 2021); and qualitative traits in sunflower (Terzić *et al.*, 2020). Extensive research activities on 2217 landraces from the Western Balkan region and 3258 introduced populations and inbred lines from 40 countries conducted at the Maize Research Institute 'Zemun Polje' (MRIZP) gene bank (Anđelković and Ignjatović-Micić, 2012) represent a successful example of pre-breeding for drought tolerance in maize. The major outcome of the investigations was the establishment of the MRIZP drought-tolerant mini core collection, comprising 15 inbred lines, 13 landraces, and 12 introduced populations (Ignjatović-Micić *et al.*, 2014; Vančetović *et al.*, 2014). Their abilities to combine with elite inbred testers were determined, which enabled the development of breeding populations with a different share of drought-tolerant and elite germplasm. Consequently, several inbred lines developed from these populations are parental components of hybrids currently being tested for registration by the Plant variety registration department of the Republic of Serbia. More recently, a molecular analysis of diversity patterns in a large panel of South-east European maize inbred lines (Galić *et al.*, 2021, Preprint) offers new opportunities to enhance the level of adaptation to regional conditions in new maize varieties.

The Pannonian region is characterized by intensive agricultural production, predominant commercial varieties, and relatively low on-farm genetic diversity. Old traditional varieties and local landraces of wheat, barley, and maize could be found only sporadically on small-scale farms. Despite the long cultivation tradition of cereals in South-east Europe and the need for preservation of traditional varieties and local landraces on-farm, there are few available data on their inventory (Dajić Stevanović and Đorđević Milošević, 2018). Similarly, a severe decline of biodiversity and the complete loss of traditional local varieties on farms in the region were also reported for industrial crops (Rivera *et al.*, 2018). Thus, improvement of biodiversity through the cultivation and use of local varieties that possess strong phenotypic and genotypic plasticity and the ability to adapt to various ecoregions along with the careful introduction of new species with inherited resilience to climate stresses is vital to the security of food systems of the region (Čanak *et al.*, 2020). Regional breeding institutions are promoting this type of activities, notably the Institute of Field and Vegetable Crops (IFVCNS) that is actively involved in the Benefit-sharing Fund of the International Plant Treaty—GRAINEFIT and International Atomic Energy Agency projects (RER5024). Activities within these projects are focused on redesigning the exploitation of genetic resources of small grains, achieving food security, combating climate change and its impacts, halting biodiversity loss, and revitalizing the global partnership for sustainable development.

Breeding strategies for climate change adaptation

Improvement of the wealth generated by agricultural and food industries is determined to a large extent by successes in the plant breeding sector through the continuous development and release of new and improved varieties/hybrids. The main plant breeding activity in the Pannonian region is carried out in a few specialized institutions, still largely supported by state and public funds. The majority of these institutions played a significant role in the agricultural development of the region by producing and selling thousands of crop genotypes created and adapted for specific conditions of the Pannonian Plain by many successful and worldwide recognized crop breeders. During a century-long breeding tradition of this region, plant breeders were facing and solving many challenges and problems in the agricultural sector. Their research activities cover an integrated spectrum of complex topics ranging from basic and methodological problems to applied research. The fundamental goal is to develop new generic plant genotypes to satisfy the needs of the present and the future, based on the internationally acknowledged plant gene pool accumulated in different breeding centres in the region and using up to date genetic, physiological, biological, functional genomic, biotechnological, plant breeding, and crop production methods.

In the context of climate change, increasing crop production under heat and drought conditions may be the greatest challenge facing plant breeders today. In general, there are three breeding strategies for stress tolerance. The yield stability strategy implies the selection of lines for high yield potential under good production conditions, then selection of those still doing well under stress conditions. The yield adaptation strategy is based on line selection only under stress conditions assuming they will also do well under better conditions, while the integrated strategy combines good stress response traits into high yield potential lines. In the past, field crop improvement through the yield stability breeding strategy has been the major choice of plant breeding institutions in the Pannonian Plain (Table 3), when drought events were rare and wet years were predominant. Under such environments, where water availability was not a limiting factor, farmers preferred high-yielding varieties/hybrids. However, this type of variety usually suffered considerable losses in drought seasons, because selecting for high yield potential does not necessarily improve performance in less favourable (stress) environments (Slafer and Araus, 2007).

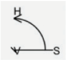
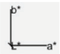
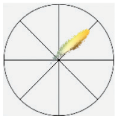
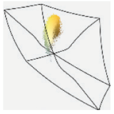
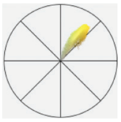
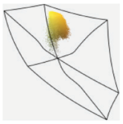
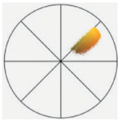
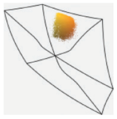
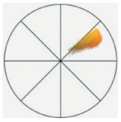
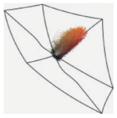
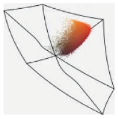
The new genetic variability under conditions of the Pannonian Plain is mainly developed through simple and complex crosses of local germplasm with the advanced genepools from Western Europe or the former Soviet Union for small grains, and Northern and Central Europe and the USA for maize. The simultaneous evaluation of wheat varieties across stress and non-stress environments showed that cultivars from Central and Southeastern Europe (Hungary, Serbia, Croatia, and Bulgaria) have high yield potential, which can still sustain a significant yield under mild to moderate drought (Dodig et al., 2008, 2012a). However, in severe drought conditions, their tolerance was mainly poor, while their yield stability was average at best. As the climate change in the region appeared to be more severe and affected yield stability, a shift in breeding strategy from yield stability to yield adaptation has become essential to create improved varieties and increase food security. In this second phase, it was necessary to find new sources of improved stress tolerance. Breeders endeavoured to identify better stress tolerance alleles/genes in landraces (Denčić *et al.*, 2000; Dodig *et al.*, 2012a; Popović *et al.*, 2020), stress-adapted genotypes (Vančetović et al., 2010, 2015; Dodig et al., 2010a, 2012b; Assenov *et al.*, 2013), and related wild species (Terzić et al., 2006, 2020; Hladni *et al.*, 2018). However, transferring abiotic stress tolerance in high-yielding genotypes by implementing the integrated breeding strategy is complicated due to a lack of in-depth understanding of the physiological and genetic basis of adaptation under stress conditions in general (Reynolds and Trethowan, 2007; Basu *et al.*, 2016). Thus, a search for the rare alleles for stress tolerance/adaptation among current high-yielding genotypes may be a more promising approach for breeding progress in the Pannonian Plain (Dodig et al., 2010a, 2012b; Zorić *et al.*, 2012; Miladinović *et al.*, 2018a; Mikić et al., 2016, 2017). Traits that are expected to be of specific benefit under water-limited environments, such as water extraction efficiency (Dimitrijević *et al.*, 2018; Miladinović *et al.*, 2019a; Blažić *et al.*, 2021), remobilization of water-soluble carbohydrates (Dodig *et al.*, 2017; Šešlija *et al.*, 2017), early vigour traits (Dodig *et al.*, 2008; Kandić *et al.*, 2009; Dodig *et al.*, 2015), crop phenology, and changes of plant architecture for better adaptation (Dimitrijević and Horn, 2018), could also improve biomass production and grain filling under all environments.

Molecular marker technologies are increasingly being used by plant breeders in the region to complement conventional breeding approaches and improve selection efficiency by identifying marker–trait associations for agronomic traits in maize (Mikić *et al.*, 2018), yield and yield-related traits, disease resistance, drought tolerance, and quality traits in wheat (Kobiljski *et al.*, 2002; Quarrie *et al.*, 2003; Dodig et al., 2010a, 2012a; Zorić *et al.*, 2012; Brbaklić *et al.*, 2015; Trkulja *et al.*, 2019), oleic acid content in sunflower (Dimitrijević *et al.*, 2017), and erucic acid content in rapeseed (Miladinović *et al.*, 2018b). Mapping of quantitative trait loci (QTLs) was employed to reveal the loci underlying yield and yield components, metal concentration in leaves, Chl *a* fluorescence parameters, disease resistance, drought tolerance, and grain quality traits in maize (Sorić *et al.*, 2011; Nikolić et al., 2012, 2013; Šimić *et al.*, 2012, 2014; Zdunić *et al.*, 2014; Galić *et al.*, 2017; Brkić *et al.*, 2020), agronomic traits in wheat (Kobiljski *et al.*, 2009), and downy mildew and broomrape resistance in sunflower (Imerovski et al., 2014, 2019). Next-generation sequencing (NGS) techniques, already successfully applied in genetic mapping and QTL analyses (Galić *et al.*, 2017), enabled genome-wide association studies (GWAS) of biomass responses to water deficit in maize (Mazur *et al.*, 2019), and phenotypic traits in wheat (Guberac, 2020). This, together with the selection of the most appropriate and effective selection strategy, such as best linear unbiased prediction (BLUP) and genomic selection (Galić *et al.*, 2019; Plavšin *et al.*, 2021), will help breeders to select parental genotypes best suited for use in local breeding programmes for better crop productivity under variable environmental conditions of the Pannonian region (Dodig *et al.*, 2010b; Nikolić et al., 2012, 2013; Zorić *et al.*, 2012).

Phenotyping for improved environmental resilience using conventional approaches

In the Pannonian region, most of the progress in major crops has been derived from classical or traditional breeding, which takes the yield as the main criterion for selection (Babić *et al.*, 2011; Miladinović *et al.*, 2015; Marjanović Jeromela *et al.*, 2016;

Table 3. Segmentation of the International Union for the Protection of New Varieties of Plants (UPOV) colour categories with Flower Colour Image Analysis (FloCIA) and colour clouds obtained by analysis with HSV (H, hue; S, saturation; and V, value of light intensity) and Lab colour space (Zorić *et al.*, 2020)

UPOV images	**UPOV images segmented**	**HSV color space, HS components** 	**Lab colour space, *ab* components** 	**Mean vectors**		
				L	***a***	***b***
				207.959	128.5699	180.9511
				220.7627	119.1718	196.9132
				194.3196	131.934	195.4371
				187.9894	147.3313	195.7025
				187.4147	151.3149	196.44
				32.28135	133.7543	131.8849
				86.7722	163.5352	155.0404

From left to right: UPOV representative images, segmented sunflower ray florets, colour clouds in HSV colour space, colour clouds in Lab colour space, mean vectors in Lab coluor space (*L*, *a*, *b*) of each colour group based on UPOV guidelines for sunflower (yellowish white, light yellow, medium yellow, orange yellow, orange, purple, reddish brown).

Cvejić *et al.*, 2019; Jocković *et al.*, 2019; Mirosavljević *et al.*, 2020a). Although breeders in the region are continuing to improve the yield potential of the major crops (Babić *et al.*, 2011; Mladenov *et al.*, 2011; Videnović *et al.*, 2013; Mirosavljević et al., 2016, 2020b; Španić *et al.*, 2017a; Zorić *et al.*, 2017), alternative approaches are being sought to supplement traditional breeding to improve performance under targeted stress conditions. One of these approaches is indirect selection and phenotyping for secondary traits, which must be putatively related to a higher yield potential and/or to improved behaviour of the crop when grown in a stressful environment. For instance, in a commercial rapeseed breeding programme, genotypes were phenotyped for abiotic stress tolerance, such as freezing, salinity, and increased heavy metal concentration, and their yield potential was evaluated in such stress environments (Oreščanin *et al.*, 2012; Marjanović Jeromela *et al.*, 2018; Jovičić *et al.*, 2019). A similar approach was used in sunflower, where phenotyping and analysis of morphological traits in both wild and cultivated genotypes were done to find morphological markers for stress resilience and other important agronomic traits (Jocković *et al.*, 2018; Luković *et al.*, 2018; Miladinović *et al.*, 2019b). Within these studies, pericarp features were identified as a potential source for the improvement of the technical and technological properties of cultivated sunflower (Jocković *et al.*, 2020).

In the Pannonian region, there are large fluctuations in the amount and the frequency of precipitation among sites within a year and, even more so, from year to year (Sudarić *et al.*, 2006; Dodig *et al.*, 2008). Phenotyping under such conditions is difficult because of the irregular and erratic drought, and consequently environmental selection pressure on breeding materials, that is changing drastically from generation to generation. To overcome this obstacle, phenotyping in controlled stress environments and rain-out shelters, which provide an experimentally controlled drought stress, has been used in several cereal crops. In wheat, >300 genotypes, including landraces, were simultaneously evaluated under fully irrigated, rain-fed, and rain-out plot shelters for yield and traditional traits such as plant height, phenology, above-ground biomass, and yield-related traits to identify parents for crossing (Denčić *et al.*, 2000; Dodig et al., 2008, 2010a, 2012a, b). More recently, Dodig et al. (2016, 2017, 2018, 2020) used leaf blade defoliation to assess the contribution and mobilization efficiency of stem dry matter reserves to the variation of grain-filling rate and grain weight in wheat and barley under drought stress after anthesis. These studies showed a similar grain yield reduction caused by leaf defoliation, which simulated a terminal drought stress by inhibiting current assimilation, to those obtained under natural post-anthesis drought stress (Dodig *et al.*, 2016; Kandić *et al.*, 2018). Strong source restriction after pollination as a tool for estimating drought tolerance was also tested in maize, and an improved breeding scheme for increased drought tolerance based on kernel properties was proposed (Vančetović *et al.*, 2019).

In order to identify critical traits to be used in breeding programmes aimed at improving the productivity under stress, a core set of 96 wheat genotypes of highly contrasting phenotypic characteristics have been phenotyped over time for >70 agronomic, morphological, physiological, and other traits (Kobiljski *et al.*, 2002; Quarrie *et al.*, 2003; Mitić *et al.*, 2009; Dodig *et al.*, 2010b), including those associated with drought tolerance at the seedling (Dodig *et al.*, 2015) and fully mature stage (Dodig *et al.*, 2012b). So far, >100 000 trait measurements have been made for 96 wheat accessions. In addition, data from 3 year trials of the 96 wheats genotyped with molecular markers to test marker effectiveness in improving breeding efficiency for increased yield under drought were used to make selected crosses. Several promising progenies of crosses were taken through to F_4 seeds (Quarrie *et al.*, 2011). Twenty-seven F_4 families along with their parents and genotypes representing other breeding material were trailed for 2 years under simulated terminal drought stress and yield, its components, and above-ground biomass, along with stem morphological (Dodig *et al.*, 2016) and anatomical (Dodig *et al.*, 2017) features, were recorded. The analyses of plant performance in controlled environments at early stages of plant development were also used by physiologists and breeders to identify tolerance-related traits or to derive stress-tolerant genotypes (Dodig *et al.*, 2015, Čanak *et al.*, 2020; Nikolić *et al.*, 2020). This approach allows not only rapid phenotyping of many lines at low cost avoiding much of the environmental noise associated with field experiments, but also screening of growth of young plants as a possible prediction for adult plant performance (Grzesiak *et al.*, 2012; Honsdorf *et al.*, 2014; Dodig *et al.*, 2015; Peirone *et al.*, 2018).

Integration of modern phenotyping tools in crop improvement of the Pannonian region

Breeding and phenotyping of major crops in the region were mainly based on traditional methodologies (measuring traits such as plant height, phenology records, biomass accumulation, and grain yield traits) that is generally time-consuming, labour-intensive, low throughput, often destructive, prone to errors in the sampling, and with a highly biased nature of the evaluation results. Recent advances in sensor technology and data processing have led to the development of less biased, high-throughput phenotyping (HTP) methods capable of capturing wide genetic diversity to facilitate the improvement of stress plasticity in crops (Reynolds *et al.*, 2020; Hein *et al.*, 2021). These promising technologies are becoming standard in agricultural research and applications. In order to assess emerging technologies against phenotyping tasks, significant steps have been made toward upgrading plant phenotyping up to the next level in the Pannonian region.

The experiences of Croatia

Maize and wheat, as the most important crops in the northern (continental) part of Croatia, have somewhat higher yields compared with other parts of the Pannonian region (Table 1). However, the projected increase in agricultural yields as a result of increasing atmospheric CO_2 concentration in Northern Europe would be counteracted by the risk of water shortage and shorter growth duration in Southern and Eastern Europe in general (Kovats *et al.*, 2014).

Breeding programmes in Croatia still mostly rely on traditional methods of phenotyping, and resources to adopt new HTP technologies are limited. However, several tools and methods (e.g. GreenSeeker, Marvin grain analyser, and the Tecan spectrophotometer) have been introduced in the last few years to rapidly and more precisely identify the available genetic variation for the traits of interest such as biomass or response of wheat plants to abiotic and biotic stresses. These tools are used for measuring the NDVI as a proxy trait for biomass and nitrogen status, seed 2D morphometry, and selected enzymes of oxidative stress caused by nitrogen and *Fusarium* sp. (Šestak *et al.*, 2018; Šimić *et al.*, 2019; Matić *et al.*, 2021). In maize, handheld chlorophyll fluorimeters were extensively used under field conditions to determine phenotypic responses of maize inbred lines *per se* to water stress (Lepeduš *et al.*, 2012). Moreover, they were used as an additional tool to assist in a QTL analysis of chlorophyll fluorescence parameters in a maize population grown in different environments (Šimić *et al.*, 2014). In a quantitative genetics and QTL study with recombinant inbred line testcrosses, a chlorophyll fluorescence analyser was used to assess photosynthetic and yield performance challenged by heat stress (Galić *et al.*, 2019). Handheld chlorophyll meter and leaf gas exchange measurements were used to determine phenotypic responses of Croatian winter wheat cultivars under terminal drought stress (Habuš Jerčić *et al.*, 2018). Besides shoot-based phenotyping under acid soil conditions, quantification of root architecture of two winter wheat varieties contrasting in aluminium tolerance was performed using X-ray microcomputed tomography (Fig. 2) (Lazarević *et al.*, 2016).

As part of the Centre of Excellence for Biodiversity and Molecular Plant Breeding (CroP-BioDiv) project, a new indoor phenotyping facility was recently established at the University of Zagreb, Faculty of Agriculture. The facility enables multispectral imaging (Fig. 3), chlorophyll fluorescence imaging (Fig. 4), 3D multispectral scanning (Fig. 5), and infrared thermal imaging. Abiotic stress affects several morphological and physiological traits in plants. Therefore, a combination of the above techniques allows for a comprehensive characterization of stress-induced shifts in plant phenotypes at the whole-plant level. The use of chlorophyll fluorescence imaging, multispectral imaging, and 3D scanning enabled discrimination between salinity and drought stress in sweet basil (*Ocimum basilicum* L.) (Lazarević *et al.*, 2021). In addition, Štambuk et al. (2021) used chlorophyll fluorescence and multispectral imaging to evaluate differences in tolerance to downy mildew (*Plasmopara viticola*) among Croatian native *Vitis vinifera* L. cultivars. Ongoing studies in Croatia focus on screening drought tolerance among common bean (*Phaseolus vulgaris* L.) germplasm, heat stress in potato (*Solanum tuberosum* L.), and viral infections in grapevine (*V. vinifera* L.).

The experiences of Hungary

During the last decade, the main aim of many research programmes in Hungary was to achieve better abiotic and biotic stress tolerance, leading to higher yield ability under the continental climate conditions of Eastern Europe. Use of modern and HTP systems for plant phenotyping under optimal and stressed conditions is essential in these studies. Having this in mind, semi-automatic phenotyping platforms were constructed in the Biological Research Centre Szeged (BRC), for monitoring shoot and root development of mid-size plants at the beginning of the 2010s. One of these platforms was installed in BRC, while two other platforms were used in the greenhouse of Cereal Research Ltd, Szeged. In 2019, new, fully automatic plant phenotyping platforms were installed in BRC for monitoring mid-size plants (wheat, barley, tomato, potato, etc.) using RGB imaging and 3D laser scanning (Fig. 6). In addition, a system for small plants (Arabidopsis, etc.) is also in operation now with a wide array of sensors (RGB, Chl fluorescence, thermal, and hyperspectral imaging), and a rhizotron system for parallel root and shoot development monitoring. The Szeged phenotyping platforms are integrated into EPPN and EPPN2020, and provide access to phenotyping infrastructures to external users, including groups from Serbia and Romania.

The BRC is also involved in the development of affordable phenotyping solutions, which can lower the significant price barrier, which currently stands as an obstacle to the wider application of phenotyping approaches. In collaboration

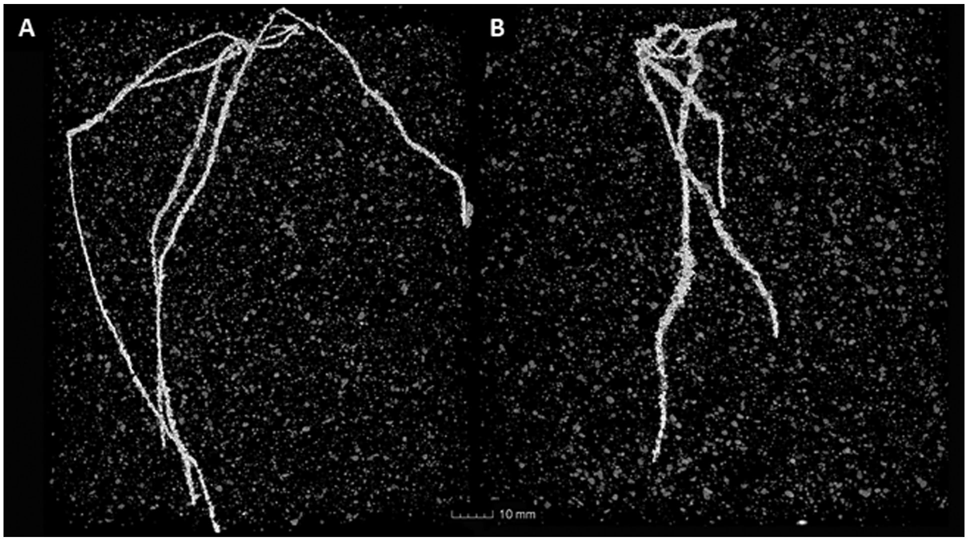


Fig. 2. Root architecture of a 5-day-old *Triticum aestivum* L. (A) Aluminium-tolerant cultivar Sivka, and (B) aluminium-sensitive cultivar Scout 66, grown in a column filled with acid soil (pH 4.6). Roots were scanned in soil columns using a Phoenix Nanotom® (GE Measurement & Control Solutions, Wunstorf, Germany) X-ray µCT scanner and were extracted and visualized by VGStudioMax® 2.0 (Volume Graphics GmbH, Heidelberg, Germany).

with informaticians of the Szeged University they have developed a cloud-based 'smart pot' system that provides a low-cost solution for monitoring plant growth in greenhouse conditions (Pflanzner *et al.*, 2020). In addition to plant phenotyping, they also developed phenotyping methods for microalgae by using precisely designed and operated photobioreactor systems with multiple sensors of physicochemical parameters (dissolved oxygen, pH, and optical density) and gas control, allowing real-time monitoring of the physiological changes and net photosynthesis under Ci limitation (Patil *et al.*, 2020).

At the Cereal Research Ltd in Szeged, three different selection levels were developed: (i) laboratory-level selection via different laboratory and *in vitro* systems (Lantos and Pauk, 2016; Nagy *et al.*, 2016; Kanbar *et al.*, 2020a); (ii) greenhouse complex stress diagnostic achievements (Fehér-Juhász *et al.*, 2014; Békés *et al.*, 2017; Nagy *et al.*, 2017); and (iii) nursery experiments carried out under rain-shed tents and in multilocation tests (Nagy et al., 2017, 2018; Kanbar *et al.*, 2020c). Using these methodologies, 10 secondary traits which could significantly discriminate high and low yield wheat ecotypes under drought stress conditions were identified, showing their importance in breeding programmes for different abiotic stresses (Abdolshahi *et al.*, 2015). Also, the drought-tolerant wheat genotypes (Kanbar *et al.*, 2020b), as well as cold- and salt-tolerant rice genotypes (Székely et al., 2019, 2021) were identified using the same approach. As a result of these consequent research and breeding activities, new wheat (GK Berény, GK Szereda) and rice (Janka, Tünde) varieties entered into cultivation (Nagy *et al.*, 2018; Székely *et al.*, 2019).

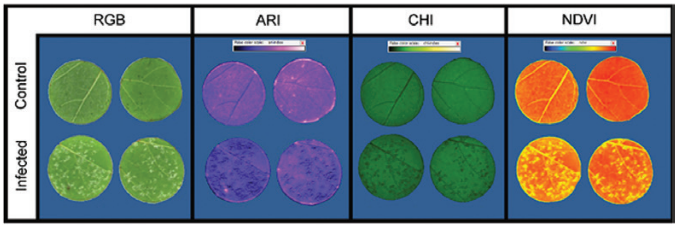


Fig. 3. Multispectral imaging and image analysis of grapevine (*Vitis vinifera* L.): control and infected (with *Plasmopara viticola* and incubated for 8 d) leaf discs. Colour (RGB) and pseudo-colour images [anthocyanin index (ARI), chlorophyll index (CHI), and normalized differential vegetation index (NDVI)] were obtained and analysed using CropReporter™ (PhenoVation B.V., Wageningen, The Netherlands).

The phenotyping installations in Szeged (BRC and Cereal Research Ltd) were also used for monitoring drought stress responses of barley (Cseri *et al.*, 2013) and wheat (Fehér-Juhász *et al.*, 2014). It was also demonstrated that grain yield and biomass are not necessarily correlated in wheat, and therefore phenotyping for biomass cannot be simply used to estimate grain yield (Paul *et al.*, 2016). A detailed study including 14 wheat genotypes of different geographic origin (Austria, Azerbaijan, and Serbia) showed that the co-occurrence of mild salinity and drought can induce large biomass and at the same time grain yield losses due to synergistic interaction of these important stress factors (Paul *et al.*, 2019).

Phenotyping studies on potato have shown that the overexpression of an endogenous annexin, STANN1, provides protection against drought stress (Szalonek *et al.*, 2015), and also that somatic hybrids of *Solanum chacoense* (+) and

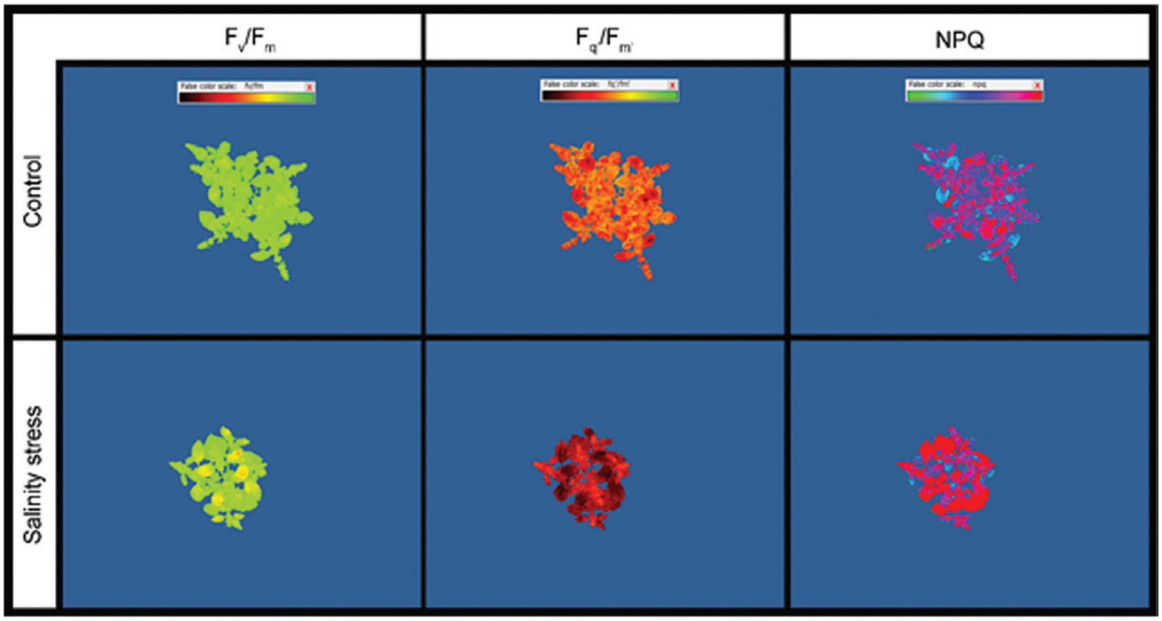


Fig. 4. Chlorophyll fluorescence imaging of sweet basil (*Ocimum basilicum* L.) grown under control and salinity stress (irrigated with 150 ml of 20 mM NaCl for 4 weeks). Chlorophyll fluorescence parameters [maximum quantum yield of PSII (F_v/F_m), the effective quantum yield of PSII (F_q'/F_m'), and non-photochemical quenching (NPQ)] were imaged and analysed using CropReporter™ (PhenoVation B.V.).

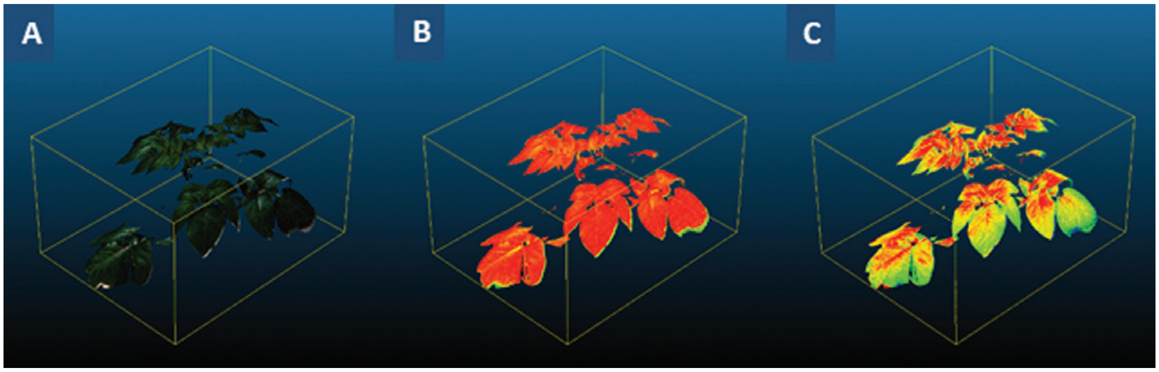


Fig. 5. Examples of 3D multispectral parameters. (A) Colour reflectance, (B) normalized differential vegetation index (NDVI), and (C) near-infrared reflectance. A 30-day-old potato (*Solanum tuberosum* L.) plant was scanned using a PlantEye F500 multispectral 3D scanner (Phenospex, Heerlen, The Nederlands). 3D plant models were extracted and analysed using HortControl software (Phenospex, Heerlen, The Netherlands).

Fig. 6. Automatic plant phenotyping system in the Biological Research Centre Szeged.

Solanum tuberosum can result in increased tolerance against drought and salt stress (Molnár *et al.*, 2021). The application of plant phenotyping methods has also been useful for the characterization of the consequences of virus infections in tobacco and tomato plants (Pesti *et al.*, 2019). A further important application of the HTP phenotyping approach was the characterization of biomass accumulation in energy willow. The results demonstrated biomass stimulation by autotetraploidization (Dudits *et al.*, 2016), chemical treatments (Kelpak, TRIA) (Digruber *et al.*, 2018), as well as increased salt tolerance in the tetraploid varieties (Cseri *et al.*, 2020).

Besides monitoring the development of the above-ground green parts of plants, it is also possible to follow root development on the same plants by using the root imaging system installed in BRC. The HAS-RSDS platform ensures analysis of root architecture parameters by using the Rhizobox and Rhizocolumn systems (Fig. 7). Rhizobox is also suitable for the detection of S-phase cells by EdU (5-ethynyl-2′-deoxyuridine) staining of the root tips. As part of the European Plant Phenotyping Networks, this platform was used in several abiotic stress tolerance projects.

Plant phenotyping is important not only under controlled greenhouse conditions, but also under natural field conditions in order to access land use and plant development on agricultural areas. The department of Physical Geography and Geoinformatics at the University of Szeged is dealing with the analysis of airborne images (RGB and hyperspectral) including evaluation of agricultural crops (Csendes and Mucsi, 2016; Gudmann *et al.*, 2020).

The experiences of Serbia

Handheld phenotyping instruments allow easier and more precise measurement of physiological parameters or their surrogates in the field. These portable instruments were extensively used by regional scientists for measurement of photosynthetic parameters in maize challenged by cadmium and salt stress (Franić *et al.*, 2018; Galić *et al.*, 2020), in wheat stressed by Fusarium inoculation (Španić *et al.*, 2017b), and during natural leaf senescence (Vuletić and Španić, 2019), as well as in barley (Begović *et al.*, 2020) and sunflower (Markulj Kulundžić et al., 2016). In Serbia, portable phenotyping including the NDVI, chlorophyll content, or canopy temperature measurements has already found a significant role in regular small grain breeding activities at IFVCNS (Mirosavljević *et al.*, 2020a).

In recent years, HTP tools and platforms have started to be more extensively used and integrated into the crop breeding programmess in Serbia. Breeding groups have been using different grant schemes and international collaborations to access modern HTP platforms through EPPN and COST Action. Through the projects of the Transnational Access to European Plant Phenotyping Network (grant agreement no. 284443), the collection of Serbian wheat cultivars has been analysed under controlled conditions using HTP equipment. In this way, the data about the salt and drought stress-induced responses of Serbian wheat cultivars, regarding their green and dry biomass, grain yield, water consumption, photosynthetic activity, and proline accumulation, were obtained (Paul *et al.*, 2019). Moreover, the genotypic response of the Serbian wheat to the effect of heat stress during anthesis and grain filling was monitored under controlled conditions by analysing photosynthesis, chlorophyll, carbohydrate, and grain yield traits using modern phenotyping equipment and providing useful information for further heat tolerance breeding under conditions of the Pannonian Plain (Mirosavljević et al., 2021a, b). In sunflower, within COST Action FA 1306: 'The quest for tolerant varieties—Phenotyping at plant and cellular level', the first attempts at sunflower root phenotyping using modern phenotyping platforms were made. In this preliminary study, one cultivated and one wild sunflower genotype were examined with the automated phenotyping platform, GROWSCREEN-Rhizo (Dimitrijević *et al.*, 2018). This work was later continued through the EPPN grant scheme.

Using the same network, a group of researchers from MRIZP conducted the experiment in which 20 diverse maize inbred lines were characterized for their responses to nitrogen deficiency and water stress, as well as combined nitrogen and water stress imposed mainly during the vegetative developmental phase. Visible, fluorescence, and near infrared imaging was performed each day, while a FluorCam device was used at two time points for kinetic chlorophyll fluorescence analyses (Dodig *et al.*, 2019). In the first study, Dodig *et al.* (2019) focused on one time point measurements, more precisely the last day of the experiment for architectural and biomass-related traits and the time of maximum water stress for colour-related traits. They identified several colour-related traits and kinetic chlorophyll fluorescence, including architectural traits related to a greater leaf area, as the most important features for biomass production ability in maize, particularly under severe stress conditions. They also managed to characterize inbred lines for their stress adaptability and stability with respect to biomass

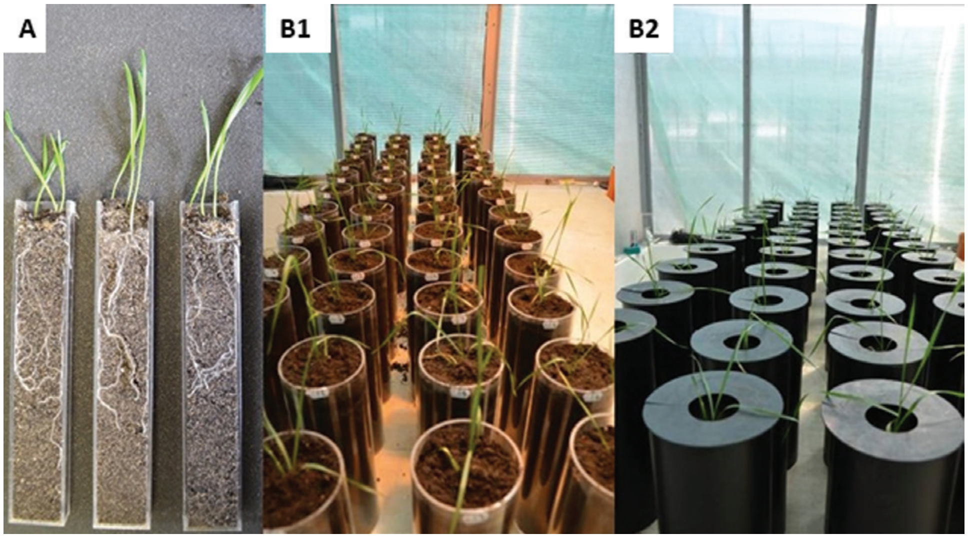


Fig. 7. The Rhizobox (A) and Rhizocolumn (B1 and B2) systems of the HAS-RSDS platform for root architecture analyses.

yield. The same HTP experiment was used to analyse genetic differences and dynamic developmental trends during different maize growth stages (Dodig *et al.*, 2021). This time, the temporal dataset included 13 biomass-related and morphophysiological traits from 33 time points. The estimated bio-volume, leaf area index, and colour ratios were found to be the most important indicators of drought adaptability, and two typical growth dynamic patterns for genotypes in water stress treatments were revealed. It was also discovered that drought recovery potential may play an equal role to drought tolerance in plant drought adaptation. Both studies contributed to MRIZP maize research and breeding for abiotic stress tolerance. However, complementary field research should be conducted to confirm the applicability of HTP in controlled conditions for extrapolation to field conditions.

The application of high-throughput technology in phenotyping rapeseed grown in Serbia is slow paced. However, at the IFVCNS, there is an ongoing pilot study on predicting seed yield and quality of rapeseed and sunflower based on data from multispectral cameras that are attached to UAVs (Fig. 8). With this means, early assessment of crop condition and the planted area is expected, which is beneficial for farmers and agricultural market stakeholders in terms of better production planning.

Thermal imaging was used in screening maize and soybean sensitivity to flame weeding, and to estimate flame temperature and temperature on the plant surface (Fig. 9). Thermal cameras acquired data on heat emission from a plant, which further led to a clue that the temperature at the plant surface is at least 10 times lower than the temperature measured in proximity to the flame (Rajković, 2018). Thermal imaging was also used for screening of sunflower for resistance to Sclerotinia head rot and Sclerotinia stem rot (Imerovski *et al.*, 2017). In the study, the contrast between unaffected and lesioned areas on thermal images was markedly higher than in visible images, potentially facilitating automated quantification (Fig. 10).

Digital image analysis is also exploited in ornamental sunflower breeding. In-house-created software enhanced colour determination of ray florets (Table 3) and paved the way to more objective and accurate sunflower phenotyping (Zorić *et al.*, 2020). They claimed that the accuracy of FloCIA software in classifying images according to colour was >90% when compared with classification executed by an observer.

Finally, an MLM is used for the prediction of soybean plant density (Ranđelović *et al.*, 2020). This research was based on analysis of RGB images taken from UAVs. After calibration, MLM was successfully validated, enabling its further use for prediction purposes in order to minimize the cost of engaging human labour.

Future prospects

Many of the crops widely grown in the Pannonian Plain today stem from a very narrow genetic base. Understanding and preserving crop genetic resources is vital to the security and sustainability of food systems both in the Pannonian Plain and worldwide. Hence, for most of the major crop breeders in the region, grain yield is the most highly valued phenotypic trait. However, to maintain crop production at its present level into the 21st century, in addition to yield, local breeders need to identify traits associated with improved adaptation, stability, and resilience in a bid to combat a multitude of environmental challenges, not just water shortage and osmotic stress. Recent advances in phenotyping tools, as well as in genetics and statistics, have provided new opportunities to provide the plant breeding sector with tools to accelerate the identification of traits of interest and the development of new improved crop varieties. However, in order to better integrate modern phenotyping tools in breeding and research activities of the Pannonian Plain region, there is a need to improve institutional capacities, to develop efficient sharing of and access to facilities, as well as data management systems that allow seamless data exchange throughout the region.

Improvement of institutional capacities

Modern phenotyping technologies and especially HTP platforms are usually very expensive and not affordable for a wide range of research institutions in the Pannonian region. However, most of the leading institutions try to adopt at least some of these tools and technologies in accordance with their financial capacities. These efforts are often hampered by lack of available infrastructure and qualified labour, as well as institutional support.

The rapid development of phenotyping technologies led to the appearance of low-cost phenotyping devices, such as low-cost environmental sensors or smartphone-embedded and mobile imaging sensors (Reynolds *et al.*, 2019), or simple greenhouse-based monitoring units. This could also provide an opportunity for research institutions in the Pannonian region, by taking into account local conditions, such as the availability and cost of labour, as well as climatic and other conditions, to develop methods and tools for more affordable and cost-effective phenotyping. It has been already shown that the investment in sensor networks for environmental characterization has a clear value for the interpretation of the genotype×environment interaction, and consequently breeding for improved resilience (Reynolds *et al.*, 2019).

Another solution for the lack of financial resources could be a closer cooperation of research institutes and breeding companies, as already implemented in some countries (Costa *et al.*, 2019b). Apart from this type of cooperation, there is a need for further support on the national and regional level through prioritizing capacity building in modern phenotyping through respective Smart Specialization Strategies and funding opportunities. Positive signs that the importance of modern phenotyping is starting to be recognized in the region are recently approved projects SmartSun (7732457) and CROPINNO

Fig. 8. Rapeseed trials recorded with a camera attached to a UAV (photo: Filip Franeta).

Fig. 9. Temperature measurement with a thermal camera. (A) Thermal imaging in soybean. (B) Thermal image (thermogram) of flame weeding machine. [Photos from Rajković (2018)].

(101059784), funded by the National Science Foundation of Serbia and EU Commission, respectively, and aimed at institutional capacity building in the application of phenomics in crop improvement.

Sharing of and access to facilities

To paraphrase Coppens *et al.* (2017) 'the future of plant phenotyping in the Pannonian region lies in synergism at the national, regional and international levels'. Hence, to meet alet all demands and high standards of contemporary research activities in this field, as well as to move agricultural science in the region to the next level, networking of the existing regional plant phenotyping facilities and joint efforts in obtaining new ones could be a possible solution for this challenge. The experiences, knowledge, and infrastructure of the European phenotyping community projects, such as EPPN2020, EMPHASIS, as well as different COST Actions, that have shown the power of connecting scientists from different fields and countries to promote the use of phenomics in crop breeding, should be exploited at the regional level. All this can help to improve scientific competence of the region as a whole, better exploitation of the facilities, as well as better coping with all the challenges of modern agricultural production and an unpredictable future.

In this regard, besides closer cooperation of research institutions, special attention should be paid to interaction and cooperation of Centres of Excellence in the region, such as the

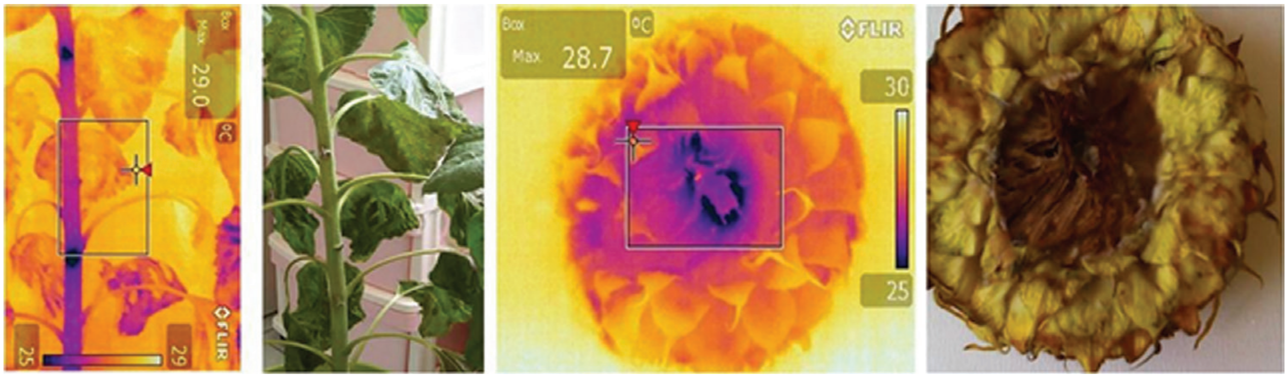


Fig. 10. Thermal imaging of sunflower reaction to Sclerotinia infection of the stem and head (Imerovski *et al.*, 2017).

CroP-BioDiv based in Croatia and the Climate Crops Centre based in Serbia, that deal with the application of modern tools in crop improvement, and that could provide a basis and support regional networking. The recently approved project, AgroServ, that aims to develop a sustainable offer of services that will deliver a wide, customized, and integrated access to state-of-the-art facilities through a common portal, involves institutions from the region and should also become a valuable tool for sharing of and access to phenotyping facilities at the European level.

Conclusions

The development of low-cost sensor technologies, along with further capacity building and networking activities, should provide new opportunities for the efficient application of modern phenotyping tools in the Pannonian Plain. By using these new tools, it will be easier for crop breeders to improve yield stability and its sustainability, to ensure better exploitation of genetic diversity present in crop wild relatives through accumulation of knowledge about their performances in abiotic and biotic stressed conditions and to address the needs of both the Pannonian countries and the region as a whole.

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Author contributions

All co-authors contributed to writing different parts of the paper; DD, DM, and AKS: editing; AKS: final adjustments and preparation of the manuscript according to the journal's instructions.

Conflict of interest

The authors have no conflicts to declare.

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