Plant Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Review article

Quantitative and comparative analysis of whole-plant performance for functional physiological traits phenotyping: New tools to support pre-breeding and plant stress physiology studies

Sanbon Chaka Gosa, Yaniv Lupo, Menachem Moshelion*

The Robert H Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, Rehovot 7610001, Israel

ARTICLE INFO

Keywords:
Plant functional phenotyping
Breeding
High-throughput
Whole-plant water balance
Simultaneously
Soil–plant–atmosphere continuum (SPAC)

ABSTRACT

Plants are autotrophic organisms in which there are linear relationships between the rate at which organic biomass is accumulated and many ambient parameters such as water, nutrients, CO_2 and solar radiation. These linear relationships are the result of good feedback regulation between a plants sensing of the environment and the optimization of its performance response. In this review, we suggest that continuous monitoring of the plant physiological profile in response to changing ambient conditions could be a useful new phenotyping tool, allowing the characterization and comparison of different levels of functional phenotypes and productivity. This functional physiological phenotyping (FPP) approach can be integrated into breeding programs, which are facing difficulties in selecting plants that perform well under abiotic stress. Moreover, high-throughput FPP will increase the efficiency of the selection of traits that are closely related to environmental interactions (such as plant water status, water-use efficiency, stomatal conductance, etc.) thanks to its high resolution and dynamic measurements. One of the important advantages of FPP is, its simplicity and effectiveness and compatibility with experimental methods that use load-cell lysimeters and ambient sensors. In the future, this platform could help with phenotyping of complex physiological traits, beneficial for yield gain to enhance functional breeding approaches and guide in crop modeling.

1. Introduction

Plants are autotrophic organisms that produce organic matter (chemical energy) using inorganic matter and solar energy. There are strong linear relationships between the rate of organic biomass accumulated and ambient quantities of intercepted solar radiation, water, nutrients and CO_2, on the one hand, and the growth rates and dry matter production of agricultural crops, on the other [1–5]. Indeed, plants' biological ability to produce is so fundamental that the word 'plant' is a synonym for factory. (According to the Oxford English Dictionary, a plant is "a place where an industrial or manufacturing process takes place" [6]).

Agricultural crops are cultivated plants that have been selected (usually through breeding programs) based on their ability to produce food, feed, fiber, fuel and other products [7–11]. The production efficiency of a crop can also be defined in thermodynamic terms, that is, as a ratio of energy output (carbohydrate) to energy input (solar radiation) [12]. In fact, the classical breeding of agricultural crops can be viewed as the evaluation of plants based on their organic matter (yield) production capabilities or their performance as "plant factories" (see Fig. 1, Table 1) and the breeding process is based on selection for the better performance of these "factories".

One of the most famous episodes in the history of agriculture is the Green Revolution, which was led by Dr. Norman Borlaug and doubled crop yields in less than 50 years [7,13]. In fact, Dr. Borlaug's success in improving the productivity of wheat by converting vegetative biomass (shorter stalks) into reproductive biomass (seeds) could be further enhanced by the application of nitrogen fertilizer without the fear of lodging [7]. This trade-off between vegetative biomass production and reproduction lies at the heart of a functional breeding.

Every year, numerous new cultivars that break existing records of yield quantity and quality are released around the world (https://mvd.iaea.org/#!Search). Nevertheless, these advances in production rates are coming too slowly to meet the forecasted global demand for food [14–16]. According to the predictions of the United Nations Food and Agriculture Organization, in order to meet global food needs, the production of staple cereal crops would need to more than double (increase by 60%–110%) over the next 32 years [14,17,18]. Conventional plant breeding, which is based on hybridization of parents and phenotypic selection of offspring, has been going on for millennia [19] and is still

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* Corresponding author.
E-mail address: menachem.moshelion@mail.huji.ac.il (M. Moshelion).

https://doi.org/10.1016/j.plantsci.2018.05.008
Received 4 October 2017; Received in revised form 14 April 2018; Accepted 14 May 2018

Please cite this article as: Gosa, S.C., Plant Science (2018), https://doi.org/10.1016/j.plantsci.2018.05.008

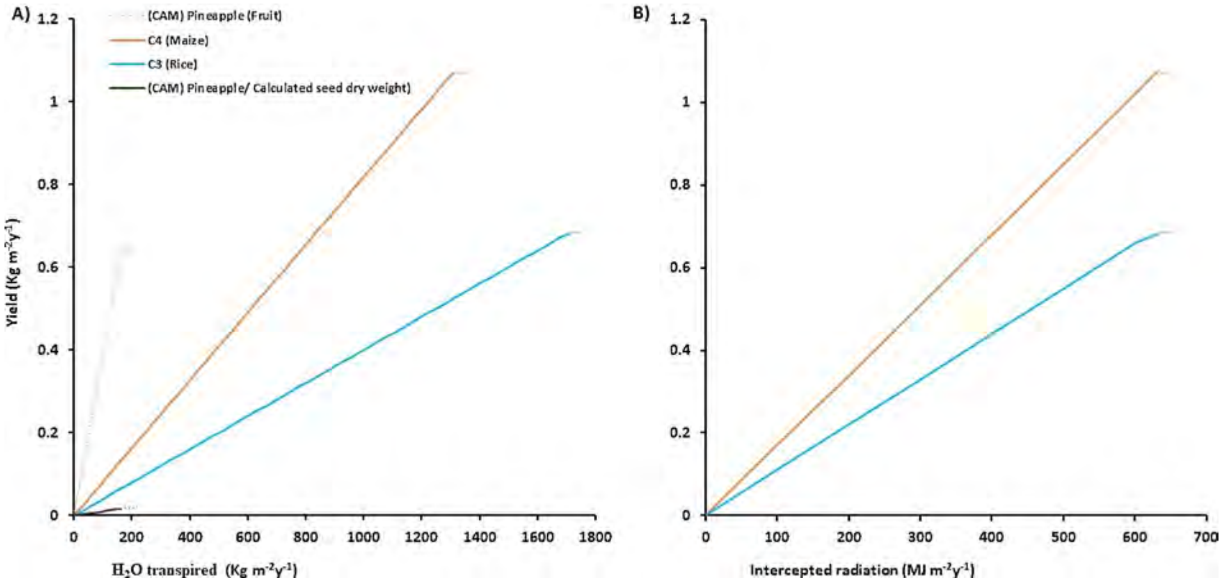

Fig. 1. The linear relationship between yield and resource consumption in different plant species. (A) The water-use efficiency of a model CAM plant (pineapple, *Ananas comosus*), C4 plant (maize, *Zea mays*) and C3 plant (rice, *Oryza sativa*). indicates fruit yield and the solid black line indicates the dry weight of seed yield estimated based on a calculation adopted for four fruit crops [35,119,120] (see Supplementary Table 1). The data shown here are modified from [12,76,121–125]. (B) The linear relationship between grain yield and radiation intercepted by foliage over the growing season for rice (C3) and maize (C4), modified from [5,122].

Table 1
Assessment of resource-use efficiency of different plant species, modified from, [5,76,121–123,128,129].

Crop Species	Maximum Yield (g $m^{-2}yr^{-1}$)	Water-Use Efficiency (H_2O g/DM g)	Radiation-Use Efficiency (MJ/ gDM)	Nitrogen-Use Efficiency (gN/gDM)
C4 (maize)	1073	1222	0.59	0.019
C3 (rice)	681.1	2497	0.90	0.037
C3 (wheat)	524.3	1827	1.46	0.023
CAM (pineapple)[a]	633.8	255	(not found)	0.024

[a] Fresh fruit weight; dry matter is assumed to be 15.2 g m^{-2}, which accounts for 24% of the fresh fruit weight (data for other crops are presented as grain dry weight).

commonly used today. While these hybrid seeds have had a tremendous impact on agricultural productivity, the breeding process is still relatively slow. An average breeding program for an annual crop can take 10–12 years[18,19] depending on the target environment [20], availability of genetic variation, the heritability of the trait of interest and the efficacy and efficiency of the phenotyping and/or genotyping methods used [21,22]. Maintaining the current rate of yield improvement in the face of climate change is challenging in itself. As a result of climate change, productivity is expected to drop by up to 50% in many parts of the world by 2080 [18]. In fact, many new cultivars have been bred under non-stress conditions and most new cultivars show dramatic yield losses under conditions of abiotic stress [23–26]. Breeding under abiotic stress is a particular challenge [27,28] and those programs that do attempt to address that challenge yield only a few new abiotic stress-tolerant cultivars each year. In a crop such as maize the commercial breeding even didn't target for stressful environment but public programs such as CIMMYT [29–31,22]. This program has managed to release drought-tolerant maize varieties (PAN 53, SC 301, PGS 61, ZM 401, ZM 521) suitable for use in different African countries [29,29–31]. Pioneer Hybrid International [32] and Syngenta [33] claim to have developed new drought-tolerant maize cultivars with yield advantages of 5% and 15%, respectively. In addition, many breeding programs focused on drought tolerance have succeeded in improving nitrogen fixation under drought stress conditions, in a manner that is not directly related to crop water use [31].

The difficulty of stress breeding can be attributed to several factors, including the complex nature of abiotic stress tolerance, the instability and uncertainty of environmental conditions and the lack of clearly defined stress scenarios, tolerance markers, and anticipated trait phenotype [34,35]. These circumstances may explain the low efficiency of programs aimed at breeding for tolerance of abiotic stress and the fact that relatively few new cultivars are released annually and that those releases follow much longer breeding periods [36].

In recent decades, the development of technologies associated with molecular markers and genomic selection has provided new molecular tools that have enhanced classical breeding processes and have made the process of breeding for simple and complex traits more efficient [37–40]. In fact, to date, genotyping has not been a limiting factor in predicting the accuracy of genomic selection [38]. The technical challenge of genomic selection in crop plants lies in the reliability of the available phenotypic data. The difference between the genotypic and phenotypic data available to breeders creates a situation known as the genotype–phenotype gap (GP gap). The anticipated improvement of genomic tools as well as other “omics” technologies (i.e., metabolomics, proteomics, and transcriptomics) is expected to yield a flood of information. As a result, breeding processes are expected to expand greatly [13,41–43]. However, we also expect to see an increase in the genotype–phenotype gap as a plant’s DNA sequence can be determined much more quickly than the knowing its function in an organism or than we can exploit identified genes for crop improvement [44]. Therefore, to harness the full benefits of new technologies, we will have to apply those technologies together with high-throughput phenotyping [36]. It is important to emphasize that the genotype–phenotype gap will be particularly complex when it involves interactions with the environment, in general, and interactions with water-limited environments, in particular.

2. Current phenotyping techniques, technologies and plant functional phenotyping for physiological breeding

Phenotyping is the comprehensive assessment of complex plant traits such as growth, development, tolerance, resistance, architecture, physiology and yield, and the basic measurement of individual quantitative parameters that form the basis for more complex traits in certain environments [45]. Traditionally, breeders have visually phenotyped their lines, subjectively and based on their personal experience. Despite its importance for the success of breeding strategies, visual selection is challenging, particular with regard to physiological trait and traits that are hard to view (e.g., roots [46,47]). Therefore, selection for root performance (e.g., in the context of salt stress) has been done mainly in hydroponic systems [46]. Those low-throughput systems involve a very artificial root environment and do not impose physical constraints on root growth. Moreover, plant-stress research, in general, and breeding for stress tolerance, in particular, are particularly complex endeavors [48]. Accordingly, there is a huge gap between the number of published papers and patents (i.e., research) and commercial stress-tolerant cultivars (i.e., practice). One of the major bottlenecks in stress-response research is the lack of a simple and fast functional screening method that would enable the selection of the desired physiological traits. As these traits are highly regulated by the environment, any screening system must involve continuous monitoring of the plant environment (soil-atmosphere), as well as plant responses to changes in that environment. In addition, it is important that the measurements be conducted simultaneously on all of the plants that are being evaluated as non-simultaneous measurements may lead to the inadvertent comparison of traits under different ambient conditions.

Physiological factors such as leaf gas exchange [49], canopy temperature and spectral reflectance have been used to investigate plant water relations. The measurement of transpiration and the calculation of stomatal conductance to water vapor are important [49] for efforts to select crops with desirable levels of these traits [47,50,51]. However, gas-exchange evaluations are limited to small areas of the leaf surface, which are used to represent the entire plant, and labor is required for the measurement of each sample [49]. Infrared imaging and other remote-sensing methods have been introduced to quantify differences in canopy temperature (CT), as well as other emitted wavelengths. Those are quick, simple and affordable methods for the selection of physiological trait selection [52,53]. While we do not discount the important contribution of these imaging phenotyping facilities, it is important to note that these measurements are affected by atmospheric conditions (e.g., cloud, wind direction) and radiation intensity and angle [47]. Moreover, to date, there is no reference library characterizing plant-emitted wavelengths in terms of plant physiological status. Therefore, the collected data need to be manually tagged in relation to the plant status. Phenotyping technologies are faced with the additional challenge of collecting precise phenotypic data in a high-throughput manner. This challenge has inspired public and private plant research institutes around the world to build phenomic facilities and the number of phenotyping facilities has increased from five before 2009 to 44 by 2015 [54]. Most of these facilities collect information in controlled environments throughout the plant life cycle using robotics and automatic image acquisition and analysis [13,19,55–57]. The most common phenotyping approaches use different wavelength-range sensors, cameras and versatile platforms (e.g., moving plants on tracks, moving sensors on cables, drones) to capture signals from plant populations. However, it is difficult to use these technologies to collect meaningful information regarding dynamic plant × environment interactions and plants' dynamic responses to water stress [55,58–60] due to the fact that plants are very sensitive to many signals from their immediate environment (e.g., light intensity, relative humidity, CO_2 concentration, soil moisture) [61]. Plant response to water deficit is highly dynamic and flexible [59,62]. Most of these environmental conditions are not stable and change rapidly even under controlled conditions. For example, plants that are exposed to drought will rapidly develop different soil water conditions, depending on their respective transpiration rates. Moreover, even under similar ambient conditions (e.g., a particular level of light in a greenhouse), measurement data will be collected from different plants at different hours and those different hours mean different light conditions due to the natural changes in light intensity over the course of the day. That means, measuring a single trait (or a few traits) on a single date across numerous genotypes will not necessarily yield insights into the plants' functioning or the genetic control of the trait in question [63], in general, or under stress conditions, in particular.

2.1. Targeting the appropriate trait and defining stress breeding goals

The definition of beneficial stress-related traits is complex and those traits must be well-defined in breeding programs [34]. Creating a drought-tolerant plant does not necessarily mean increased productivity. This fact is demonstrated in the previous study [64], who reported that while altering the expression of regulators of drought responses has often succeeded in enhancing drought tolerance, at least under laboratory conditions, that tolerance typically comes at the expense of inhibited development and carries a significant yield penalty. Similarly, breeding for enhanced water use can lead to a yield penalty [65]. Although improved yield potential can be translate into better performance under stress, it also places a greater demand on water resources and other resources [66]. In fact, modern crops use an immense amount of water due to their high rates of transpiration (see Fig. 1, Table 1), yet their high levels of stomatal conductance limit their ability to survive under water stress [67]. In order to improve crop drought tolerance, it is critical to strike the appropriate balance between defining clear breeding objectives and ensuring flexibility within the breeding strategy [68,69]. One of the common mistakes in crop breeding is to relate to survivability traits as tolerance or resistance traits. In fact, survivability traits are less relevant, assuming that the main goal of the breeding program is to improve the crop's yield under abiotic stress conditions, relative to an unstressed control (i.e., selecting genotypes for which such conditions carry the smallest yield penalty) [70]. In these cases, physiological traits related to plant water relations are the most relevant traits [34,65,71] to select for yield, as they lead to smallest yield penalty. Unfortunately, the major physiological parameters reported by imaging-based frameworks are related to plant size [72]. This situation does not provide a comprehensive, real-time, accurate description of the plant's physiological status, in general, or its water-balance status, in particular in relation to real environmental cue such as radiation,VPD and temperature. For example, maize crop sensitivity to VPD has steadily increased over recent year and as a result the current yield loss would be up to 15% which could reach up to 30% if the sensitivity trend continue in the future [72]. In fact, accurate physiological phenotyping of well-defined specific traits is essential for moving crop breeding forward [55].

3. Plant water consumption and yield

For photosynthesis, a plant needs to absorb carbon dioxide from the atmosphere, but doing so exposes it to water loss, leading to the threat of dehydration. To be productive, leaves need to maintain a well-balanced hydration status [73]. Slight imbalances in the flow of water will lead to water deficits and severe malfunctioning of many cellular [11] and physiological processes [74]. The regulation of this water balance is always challenging, particularly under water-limited conditions (see Table 1). In general, for every gram of organic matter they produce, C3 crop plants absorb approximately 500–600 g of water through their roots and that water is transported through the plant body and lost (transpiration via the open stomata) to the atmosphere. (In reality, these numbers reach 50–80 tons of water per hectare per day, as estimated based on average crop species at flowering stage [75]). C4 crops are more efficient with "only" a 1 to 250–300 g ratio [75,76] (Fig. 1, Table 1). This is three to five times greater than the amount of water used by CAM plants; those plants use 50–100 g of water to produce 1 g of dry matter [75,76]. Nevertheless, despite the greater abundance of CAM plants in the plant kingdom, as compared to C4 plants (Fig. 2A), their contribution to the global gross primary production (GPP) is very

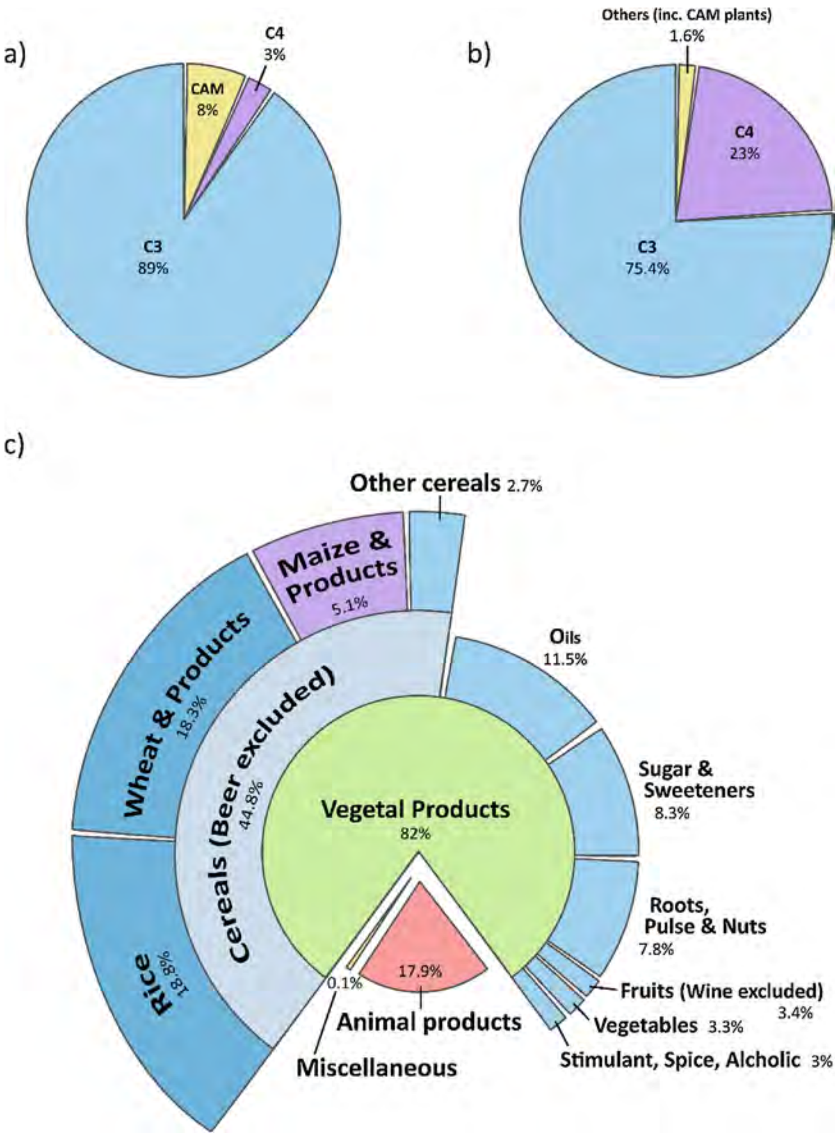

Fig. 2. (A) The distribution of CAM, C4 and C3 plants among the 250,000 species of terrestrial plants [126] and (B) their respective contributions to global gross primary production (GPP) [127]. (C) The proportions of the global dietary energy supply, kcal/capita/day supplied by the different species [122]. The inner pie chart shows the proportion of global kcal/capita/day provided plant products as compared to animal products. The middle pie chart shows the cereals' share of the total contribution of products to the global dietary energy supply and the outer pie chart shows the relative contribution of the three major cereals. Although maize is the cereal produced in the largest quantities, its contribution to global kcal/capita/day is below that of wheat and rice since a great, deal of the maize that is grown is used for feed (see Supplementary Table 2). The presented data are estimates based on data from [122]. The category “other cereals" includes barley, rye, oats, millet and sorghum.

small (Fig. 2B) and their contribution to the global dietary energy supply is negligible (Fig. 2C). On the other hand, C4 plants make a greater (more than 7 times) contribution in its gross primary productivity (GPP) compared with their abundance in plant kingdom. These C4 trends are mainly the direct outcome of breeding strategies and are logical considering the yield capacity and resource efficiency of these plants (Fig. 1 and Table 1). C3 cereals and other crops account for the majority of global plant-based daily caloric intake (Fig. 2C). The majority of our food crops exhibit lower levels of water-use efficiency and this implies that there has been a breeding preference of high yields over high water-use efficiency. Future breeding programs for improved tolerance of water stress should take into consideration the fundamental tradeoff between plant productivity (high water usage) and survivability (low water usage), which must be well-characterized, defined and addressed in stress-breeding programs, in order to improve the selection of crops that actually use water efficiently [65]. In order to do so, new phenotyping tools that involve direct and continuous measurements of plant water relationships will need to be integrated into breeding programs.

3.1. Current tools and technology for direct measurements of plant water relations

Since plant water balance-regulation mechanisms have a tremendous impact on plant stress responses, productivity, and resilience [34,62,71,77,78], it is likely that these physiological traits will be integrated into abiotic stress-breeding programs. Unfortunately, until recently, the measuring tools available to breeders were very limited and included mainly low-throughput and complex tools for the evaluation of plant water status.

3.1.1. Leaf gas-exchange measurement

Typically, stomatal conductance, transpiration, photosynthesis, and water status are measured manually. All of these traits can be measured using the stable isotopes ^{13}C and ^{18}O [79,80], and leaf gas exchange chamber. The ^{13}C method is used to assess intrinsic water-use efficiency, which is the ratio of photosynthetic carbon fixation to stomatal conductance; whereas the ^{18}O method is used to measure the amount of water taken up by the plant, evaporative and diffusional effects in transpiring leaves and isotopic exchange between oxygen atoms in organic molecules and plant water. The leaf gas exchange measurements are instantaneous and nondestructive. However, these are not high-throughput techniques and only a very limited number of plants can be measured at each time point (i.e., there is a limit to the number of simultaneous measurements that can be taken under similar ambient conditions) [81]. Moreover, a well-trained researcher must operate the measurement machine the whole time. In addition, the continuous presence of the user near the plants may modify the microclimate, (e.g., CO_2, humidity, light and temperature) in the proximity of the measured plant. Attention should also be paid to the fact that single-leaf measurements may be misleading due to variability in ambient conditions, leaf age, interference of the measuring apparatus and other factors [81–84]. Most importantly, the dynamic responses of plants' water relations to the environment (hourly, daily and/or seasonally; see Fig. 5) are extremely hard to capture using manual physiological apparatuses [59], making the comparative evaluation of plant populations in a dynamic environment is very challenging. In recent years, the continuous collection of multiple types of measurement data using gravimetric load cells, as well as other ambient sensors has gained popularity as those systems offer good solutions for many of the problems raised above.

3.1.2. Weighing lysimeters (gravimetric load cell systems)

Lysimeters are one of the oldest and most commonly used tools for continuous and direct measurement of plant evapotranspiration [85,86]. Lysimeters are one of the most accurate of all of the existing methods for studying crop water requirements [87]. Nevertheless, until recently, the simple load cell lysimeter method faced significant challenges that prevented it from being used as a high-throughput screening method. These challenges were mainly related to the technical problems of distinguishing changes in soil weight from changes in plant weight (i.e., biomass gain). Other problems were related to differences in plant size and ambient conditions (i.e., normalizing the measurements to plant size and ambient conditions was challenging), which are very important due to the temporal and the special nature of the comparative experiments. Other issues are related to technical problems of maintaining precise soil water content levels (for all of the plants throughout the experiment), pot size effect (build-up of salinity in the soil due to irrigation) and the complexity of applying different treatments to many plants simultaneously (e.g., salinity gradient, differences in soil water content or individual stress and recovery periods for each plant based on its own unique performance). However, a recent study (published as a technical advanced issue of the *Plant Journal*) [59] reported the solving of all of these problems through the use of a simple and effective experimental platform. The authors of that work reported on a high-throughput system for simultaneous and continuous monitoring of water relations in the soil–plant–atmosphere continuum (SPAC) of numerous plants under dynamic environmental conditions. Their system provides a simultaneously measured, detailed physiological response profile for each plant in the array over time periods ranging from a few minutes to the entire growing season, under normal, stress and recovery conditions and at any phenological stage [59]. The authors installed additional soil and atmosphere sensors for each pot in the array and a specially designed algorithm enabled the detailed characterization of whole-plant transpiration, biomass gain, stomatal conductance and root flux. They also enabled quantitative calculation of the whole-plant water-use efficiency and relative water content at high resolution under dynamic soil and atmospheric conditions. In fact, this functional-phenotyping platform opens new opportunities for classical breeding in functional-breeding programs, using multiple physiological traits as selection markers [34].

4. Functional physiological phenotyping and its principles

Plant traits that play critical roles in responses to environmental conditions are called functional traits. These include traits involved in morphological, biochemical, structural, phonological and physiological processes such as photosynthesis and transpiration [88]. Plant response to the environment, in general, is very dynamic spatially and on hourly basis [89–91]. To understand the variation in these complex traits, statistical approaches and models have been developed based on functional mapping and functional genomics and those tools have provided us with a great deal of information on individual cellular components at different developmental stages [90,91]. However, whole-plant stress responses are not yet fully understood [90]. Therefore, integrating the knowledge and information gathered through functional mapping and functional genomics with functional physiological phenotyping will give us an opportunity to better understand plant stress responses over time and in changing environment. Functional physiological phenotyping (FPP) is physiology-based, high-throughput, non-destructive and non-invasive phenotyping technique that continuously measures the plant and its ambient conditions (soil and atmosphere). FPP uses fitting models to characterize a plant’s response to treatment conditions and compare that response to the response of control plants measured simultaneously [59]. The comparative statistical analysis of each plant’s dynamic performance curve in relation to the entire population provides a detailed behavioral profile (under similar or different treatments), which can be used to select the best-performing plants under specific conditions or to better understand the biological mechanisms controlling their response. The accuracy of FPP enables the detection of small changes in specific physiological traits associated with environmental changes and the statistical analysis provides tools for the selection of plants that exhibit desired behavior, in relation to control plants. For example, the plant that maintained the most similar behavior to its non-treated control under conditions of salinity or drought will be considered to be the most tolerant of that particular type of stress. The comparative kinetics of simultaneously measured plants could also provide good estimations of yield and, therefore, could be incorporated into functional breeding programs (particularly at the pre-breeding stages). In addition, FPP produces unique and valuable information for applications in physiology, plant nutrition studies and other areas of importance for agronomic management. One of the most promising aspects of the FPP technology is its ability to perform whole plant performance analysis for hundreds of genotypes at the same time under certain environmental conditions [34] with different stress scenarios. Drought stress are clearly defined based on 'theta crit', controlled in a manner relevant to target environment conditions, provide quantitative knowledge and precise phenotyping. Nevertheless, in using pot-based FPP platforms, caution must be taken to prevent experimental artifacts that can result from the pot effect (see Table 2). Most of these artifacts can be eliminated, to ensure highly accurate results similar to those obtained from work in a gas-exchange chamber as was demonstrated by [57]. In addition, the use of a randomized experimental design with several repetitions is a very effective way of reducing the environmental effect. However, by no means will pot experiments replace field trials. Field trials are the best way to evaluate drought tolerance through direct measurement of the optimal trait, for example, grain yield, under drought conditions. Therefore, after pre-field screening, physiologically superior genotypes must be tested in the field.

4.1. FPP platform for root functional phenotyping

The main role of plant roots is to supply water and minerals to the plant under all conditions, including those that are stressful [92–94]. Root water flux, in general, and root performance, in particular, are important physiological parameters to be considered in crop-improvement programs. Therefore, breeders consider root-system architecture and function to be important traits for selection [27,94]. Recently, it was reported that a sensor-based FPP platform can be used to phenotype these complex traits."

4.1.1. Root phenotyping based on root flux

Soil moisture probes are one way to monitor root flux. TDR sensors can be used to monitor root flux, as demonstrated in a study in which

Table 2
Putative pitfalls of pot experiments and a gravimetric system and suggestions of ways to reduce experimental artifacts.

Source of variation/error in pot-growing experiments (the pot effect)	Explanation	Eliminating the pot effect
Small pot size	Low water content, rapid changes in soil conditions	Use of different sizes of load cells and adaptors to enable the use of larger pots
Salinity build-up	Pots are frequently fertigated. Unflushed soils lead to increased salt concentrations in the pot.	Flushing with drainage (e.g., irrigating to more than the field capacity to wash away the excessive salts).
Water loss by evaporation	Water is lost to the atmosphere from the soil surface.	Covering the soil surface
Relatively high soil temperatures	Soil in pots exposed to the sun reaches a relatively high temperature due to the relatively small volume of soil in each pot.	Use of isolated pots, reflecting coolers.
Heterogeneous growth medium between pots	Great variation in soil composition, compactness and moisture content between pots	Bulk soil mixing and homogenization before pot-filling
Drought definition	Users define drought subjectively and differently (based on time, soil sensors, soil weight).	Drought stress defined in terms of soil water limitation, Θ_{crit} for each plant based on its performance
Wide variation in the exact treatments between pots; difficulty replicating treatment conditions	Stress is applied manually with no feedback regulation to maintain the desired level throughout the experiment. The soil conditions for each plant vary based on that plant's transpiration rate.	Simplified stress scenarios using controlled-feedback irrigation systems can be used to apply multiple treatments and maintain the desired stress level automatically.
Heating of lysimeter surface	Load cells exposed to direct sun overheat, resulting in an increased noise-to-signal ratio.	Protecting the lysimeter plate from the sun with a proper cover, use of temperature-compensated load cells
Soil compaction due to non-uniform irrigation	The fall of water from a dripper located above the soil, at one point, increases the compactness of the soil at the location where the drops fall, creating heterogeneous soil wetting column.	Use of a split dripper system that allows the water to penetrate the soil in multiple locations
Excess water on load cell	Water drops from the pots are trapped on the load-cell surface (and under the pot).	Draining accumulated water from the load cell

12 sensors were installed in the soil surrounding roots, to monitor any spatial or temporal change in the distribution of moisture in the soil [83]. These soil-moisture probes are integrated into the FPP platform to continuously and precisely measure the rates at which water flows into the roots (Jr) of individual plants [59]. The flow rate is measured at the same time as other environmental signals and physiological parameters, to allow for the comparison of multiple functional traits. In addition, the continuous measurement of plant transpiration and canopy conductance by a lysimeter sheds light on the temporal difference between both root influx and shoot out-flux [59], enabling the calculation of the whole-plant relative water content (RWC), which serves as a benchmark for plant water status.

4.1.2. *Functional root phenotyping based on Θ_{crit}*

A recently published article [59], described a FPP that has been set up to work with a model constructed to identify the critical theta (Θ_{crit}) point at which soil water content becomes a limiting factor for plant transpiration [34,57]. Θ_{crit} is an important parameter for efforts to identify plants' water-balance behavior and the ability of roots to take full advantage of any available water may be a significant component of Θ_{crit}. Therefore, among plants with similarly sized shoots and similar transpiration levels, those with better root-performance capabilities (which may be the result of root architecture, anatomy and/or biochemical or physiological mechanisms) will exhibit lower Θ_{crit} values. That is, the plants with superior root performance will be able to maintain their normal transpiration rates in drier soils (see Fig. 3).

4.1.3. *Phenotyping based on the root daily water absorption rate*

The FPP system can also be used to identify high-performance rootstocks based on root daily flux rates, as reported by [95]. Those authors revealed that tobacco plants over-expressing an aqaquaporin exhibited better root permeability under salinity stress [95]. When the transgenic root was grafted onto a control plant, there was an increase in the plant's rate of stomatal conductance under both well-irrigated and saline conditions (see Fig. 4). As a result, those grafted plants exhibited increased photosynthesis and yield. This is due to the moderation of root hydraulic conductivity, an important physiological parameter that can be measured using the FPP platform.

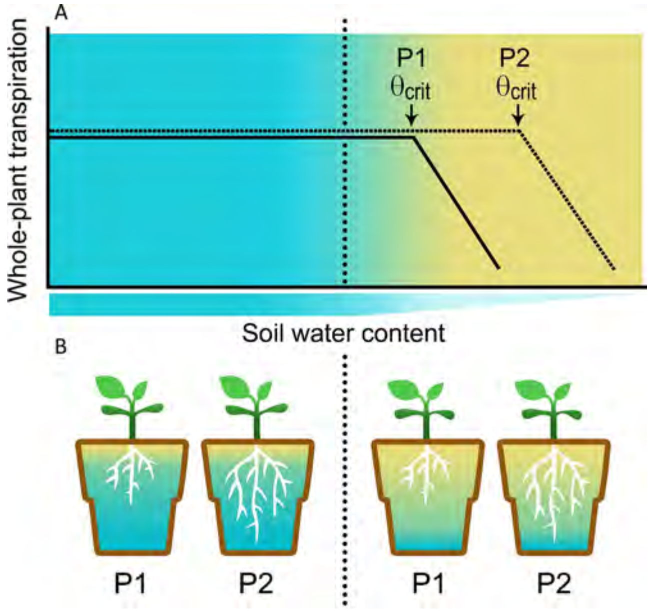

Fig. 3. Θ_{crit} as root functional trait to select high root system performance. Under 100% soil water content where water is not a limiting factor (Fig. 3(A), thus plant 1 (P1- low performance root system) and Plant 2 (P2 – High performance root system) do not limit their canopy demand for water (as revealed in similar whole plant transpiration performances). Under water deficit (Fig. 3(B), water becomes less available to the roots, thus P1 plants will be limited faster (early Θ_{crit}) than P2 plants. this approach may be highly useful to select root stocks for grafting.

5. Physiological traits functional phenotyping for plant breeding

Now days, applying physiological strategies in crop breeding to improve yield potential both under normal and stress condition is recommended [53]. The key steps and strategies for precise physiological breeding, has been suggested [47], in which high-throughput and precise phenotyping is required for parental and progeny selection from a genetic resources and after physiological trait oriented crossing respectively. In this strategy, fast screening methods for early generation is very important to enhance the rate of breeding process [53].

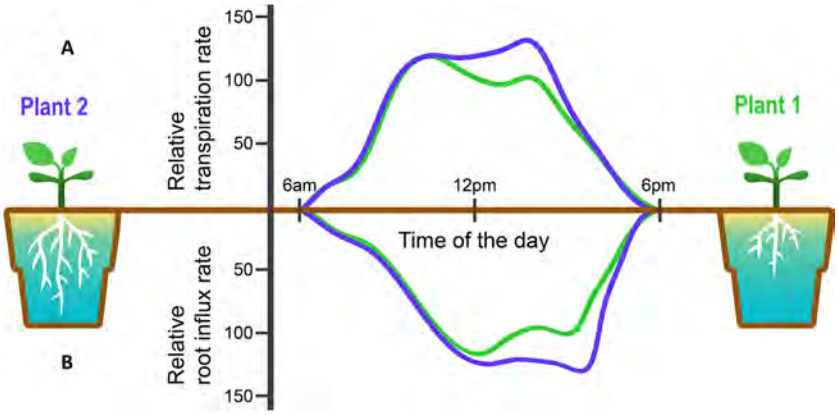

Fig. 4. Root performance impact on shoot transpiration. Representative example of the difference between plants with good (Purple line, plant 2) and poor (green line, plant 1) root performance under salt stress. A) Differences in the daily transpiration rate. B) Daily root influx (rate of water absorption). Note that the transpiration rates of the two plants are similar until late morning and converge again in the late afternoon. The midday decreases in root influx and the rate of transpiration are related to the roots' inability to support the shoots' transpiration (Modified from [59,95]. Sade et al. showed that this increase in midday transpiration (repeated daily so that there is cumulative effect for the season) translates into significant increase in the fresh weight of tomato fruit as well as its harvest index.

Morphological signs of stress may appear when it is already too late to take any action. Therefore, we think that highly sensitive physiological screening for stress symptoms at an early stage of crop growth may provide us with good indications of the well-being of the plants for breeding program. For this purpose, we believe that the FPP approach will help breeders to at least partially understand how plants can adapt to water-deficit conditions, or how different genotypes can respond differently to the same stress, and will be useful for the early screening of parent genotypes, inbred lines and crosses. Moreover, using FPP to quantify daily biomass gain and transpiration efficiency as part of the screening procedure will help breeders to select lines with desirable traits to be used as parental lines in breeding programs [10]. This will lay a foundation for stress breeding based on a physiological approach by informing breeders' which combinations of traits or alleles would best improve yield in target environments, for decision making to discard physiologically inferior genotypes at early generation stage before going to field for yield test which is economically feasible. Breeders may benefit from the early prediction of beneficial trait and improve their selection of candidate genotypes for parental crosses (F1 stage), as well as the selection of lines and crosses to be included in more advanced stages of the breeding process.

In previous studies, significant genetic gain has been demonstrated for many physiological traits in wheat breeding for drought stress [96]. This implies that integrating more functional physiological traits into the process of parental selection and the evaluation of offspring at different stages of the breeding process will enhance the efficiency of breeding programs, particularly those focused on plant–environment interactions and crop performance under sub-optimal conditions. Traits such as Harvest index (HI) [50], radiation use effeciency, early flowering, and an increased rate and duration of kernel filling, are considered as a physiological traits which increase the potential yield of wheat in certain environments [97]. We suggest that focusing on the quantitative physiological traits [48]such as $A_{N,}$ g_s, whole plant water balance, transpiration efficiency, leaf hydraulic conductance, and root hydraulic conductivity may benefit future breeding program.

The following physiological parameters have been reported to be highly correlated with yield in many crop species: stomatal conductance [98–100], hydraulic conductance [93,100,101], growth rate [102,103], anisohydric behavior [71,104–106], efficient water use [65], membrane stability index (MSI) [107,108], total chlorophyll content [107,108], low midday water potential [109,110], low midday relative water content [107,108] and transpiration-limited traits (TR_{lim}) [70]. These dynamic traits are affected by natural changes in environmental conditions. Even under well-irrigated conditions, the natural daily changes in light and VPD have a major impact on some important traits such as stomatal conductance and transpiration rate (as we demonstrated in Fig. 5). The fact that FPP could be used to measure many of these traits (see Table 3) continually and compare many plants simultaneously enables us to detect small changes during certain parts of the day in the response to environmental changes. Small differences in the way these changes occur in different plants, if repeated on a daily basis, may have significant cumulative effects on seasonal production.

In addition, measuring the currently identified quantitative physiological traits, we think that exploiting and accessing new beneficial physiological traits is very important. One example of a single physiological trait with good potential for improving plant productivity, even under stress conditions, is the "stomatal conductance golden hour". Daily stomatal conductance patterns include an early morning peak that declines as VPD increases and reaches a plateau during the late morning and midday hours [59,111]. This behavior is assumed to enable high CO_2 absorption while transpiration is very low due to the relative low VPD. In order to demonstrate this golden hour, we continually monitored whole-plant stomatal conductance, light intensity, VPD and transpiration under well-irrigated and water-limited conditions (Fig. 5). Under well-watered conditions, stomatal conductance increased sharply as soon as the light in the greenhouse increased and kept increasing until it reached a maximum level at ~07:30 (Fig. 5A). During that period, VPD was very low and in fact similar to the VPD levels seen at night values, while the light intensity reached ~40% of the daily maximum (the average light intensity during this period is 185 $W\,m^{-2}$) and the transpiration level was very low (0.33 $g\,hr^{-1}\,g_{plant}$), which is about 57% less than its maximal value at noon, which was (0.7533 $g\,hr^{-1}\,g_{plant}$). In fact, during this period, the average light intensity was 5 times greater than the minimum intensity needed to induce net photosynthesis (43.2 $W\,m^{-2}$) [112]. During this golden hour, plants are both highly productive and highly efficient. Interestingly, this maximizing of stomatal conductance under lower VPD conditions is the same strategy used by CAM plants. Moreover, this stomatal conductance peak, although reduced, is clearly observed under mild and even severe stress (Fig. 5B and C). If plants with even better use of this "golden hour" (i.e., higher and or wider stomatal conductance peaks) could be found, that could contribute significantly to plant productivity, in general, and under stress conditions, in particular.

6. Conclusion and future perspectives

About five decades ago, Donald coined the term ideotype (physiological trait-based approach) to describe a biological model that is expected to perform in a particular manner under particular conditions, namely, to yield a greater quantity or quality of useful product once developed as a cultivar [113] (Fig. 6A and B). The concept, approach and ambition to create these ideotypes for future climate change does exist [114,115], but the actual plants do not and the characteristics of current cultivars are shown in (Fig. 6C and D). This is because most of the physiological traits suggested are not considered in traditional breeding programs [70,116], due to less acceptance from breeders [117] and a lack of precise screening techniques [70]. Proposed

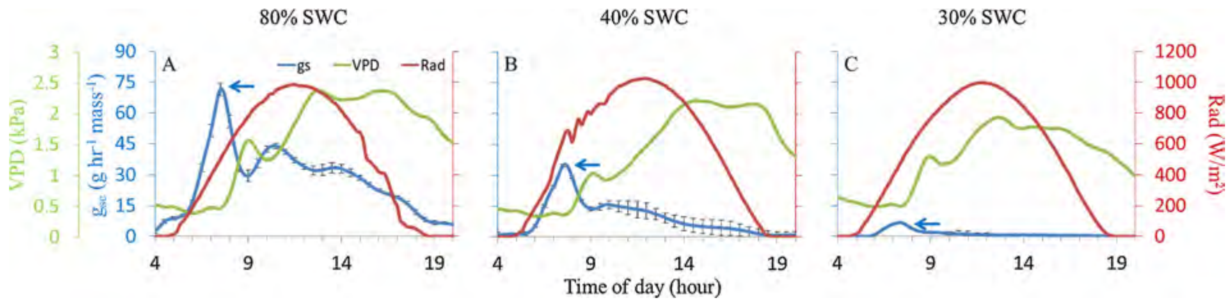

Fig. 5. Comparison of whole-plant normalized stomatal conductance of M82 tomato plants at different soil water contents. (A) Whole-plant daily canopy stomatal conductance (g_{sc}) at 80%, (B) 40% and (C) 30% soil water content (SWC); the mean of 9 plants ± SE every 30 min for g_{sc} is shown in blue (if not visible SE is < 0.42). In each graph, is the VPD (green) and radiation (Rad; red) are from the same day for the g_{sc}. Mass = plant weight (g). Blue arrow points the peak of the daily $g_{sc.}$ Measurements were taken using commercial temperature-compensated load cell (lysimeters) and dedicated sensors (Plantarry 3.0 system, Plant-DiTech, Rehovot, Israel http://www.plant-ditech.com), as described previously [59]. Plants were grown in 3.9-L pots, in a partially controlled greenhouse in which the temperature was moderated by fans cooling a wet mattress and the plants were exposed to natural day length and light. Each pot was sealed to prevent evaporation from the surface of the growth medium. The weight output of the load cells was monitored every 3 min. Whole-plant transpiration was calculated as a numerical derivative of the load cell output following a data-smoothing process. The plant's daily water loss rate was normalized to the total plant weight to get the plant transpiration rate. Continuous stomatal conductance of the whole canopy was calculated by dividing the whole-plant transpiration rate by VPD.

Table 3
List of parameters and traits for continuous and simultaneous measurement with FPP platform (based on the PlantArray diagnostic platform, as described in [59,130].

Whole-plant measurements	Environmental (meteorological) measurements
Daily biomass gain	Relative humidity
Daily water loss	Temperature (soil and atmosphere)
Water-use efficiency	Soil moisture content
Transpiration rate	Soil electrical conductivity
Canopy stomatal conductance	Vapor pressure deficit (VPD)
Root water flux	Radiation
Whole-plant relative water content	
Whole-plant water-balance	
Soil theta crit. (Θ_{crit})	
Individual stress indices	

ideotype traits such as superior performance, high productivity per day, high photosynthetic ability and high productivity per unit of water [118] are very difficult to measure in real time. Integrating FPP into breeding programs (particularly at the pre-breeding process of parental selection) will smooth breeders' journeys toward stress ideotype breeding.

In conclusion, identifying physiological markers for simple, efficient selection of parental inbred lines and hybrids could have great importance for efficient breeding processes and facilitate breeding for stress ideotypes. These physiological markers should be easy to measure quickly and heritable.

Functional phenotyping is suggested as an optimal method for stress research and breeding for the following reasons:

- It provides critical physiological and environmental parameters, which enable breeders to compare plants' dynamic responses to different ambient conditions in dynamic environments.
- The detailed performance analysis provides the researcher with quantitative tools that can be used to select and/or better understand plant mechanisms.
- In future, this mechanistic approach and the linear relationships between crop productivity and many physico-chemical factors may facilitate the development of efficient algorithms for crop modeling, early yield predictions and the prediction of yield penalties.
- It may address the relationship between quantitative physiological traits and gene actions and interactions and elucidates rules for the translation of genetic variation into phenotypic variation.
- By integrating the concept of functional physiological phenotyping with functional mapping, functional genomics provides fertile ground for the development of improved breeding strategies and platforms.

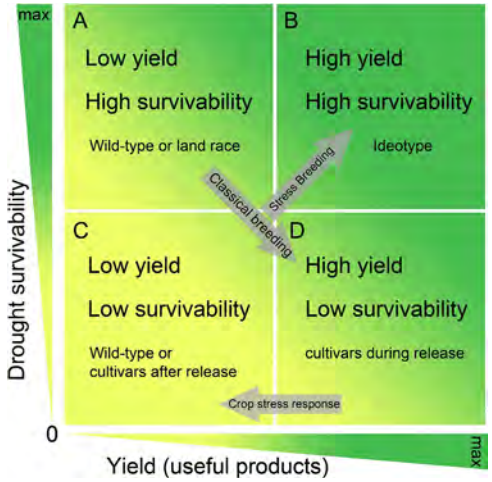

Fig. 6. The yield survivability trade-off scheme for wild-type plants and crop plants. This figure presents four possible plant productivity and/or survivability responses to environmental conditions. Each square in the figure represents an average yield capacity/attainable yield and survival rates of typical crop plants grown during several consecutive seasons, which fairly represent the distribution of the environmental conditions at a particular location. (A) Plant presenting a good survival rate and low yield-production capacity. This behavior is typical of a wild type that has evolved to sustain itself and produce viable seeds and land-race crops, which are locally adopted or traditional varieties, which do not necessarily produce their full theoretical yields. (B) An ideotype crop plant that is highly productive under any environmental conditions and can tolerate a variety of environmental changes with no significant yield reduction. (C) A susceptible plant (either wild type or land race) that cannot survive the fluctuations and uncertainty of environmental conditions between seasons. (D) A typical crop plant that was bred using a "defect elimination" or "selection for yield" approach under optimal growth conditions, but has become sensitive to unfavorable conditions. Human intervention and manipulation of wild type and land race plants are represented as moving from A to D. This trade-off between yield and survival has made many modern crops more susceptible to abiotic stress (i.e., under stress, plants shift from D to C). In an attempt to prevent this shift, current stress-breeding efforts are focused on achieving ideotype plant behaviors that will produce higher yields (useful product) even under stressful conditions.

Acknowledgements

This research was partially supported by the United States Israel Binational scientific foundation (BSF; grant # 2015100) and partially supported by the Israel Ministry of Agriculture and Rural Development (Eugene Kandel Knowledge Centers) as part of the Root of the Matter – The root zone knowledge center for leveraging modern agriculture.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.plantsci.2018.05.008.

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