

The dichotomy of yield and drought resistance

Translation challenges from basic research to crop adaptation to climate change

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The human population is increasing and so does the demand on food production. The Food and Agriculture Organization of the United Nations (FAO) predicts that in order to meet the global food demands by 2050, the production of staple cereal crops must be doubled at least (FAO, 2017), which means that the current rate of yield improvement needs to increase by at least 40%. Crop breeders are expected to cope with this challenge and come up with novel high-yield varieties, but the prospects of even maintaining the current rate of yield improvement in light of climate change are unclear. To meet the growing demand for food and increase the yield of staple crops, we need a better understanding of how plants adapt to environmental factors that limit their productivity in terms of turning sunlight and CO₂ into tissues and seeds.

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Although nature provides many examples of how plants adapt to harsh environments, these are rarely suitable for use in an agronomic environment, mainly owing to the economics: Any stress-tolerance variety must also be profitable for the farmer. If a stress

response mechanism enables the plant to survive but reduces yield, it will not be economical and, therefore, not be used by farmers. Thus, understanding the key parameters limiting crop yield—plant–environment interactions, in particular—will help us to cope with the food security challenges presented by changing environmental conditions. In particular, this knowledge helps to inform breeding programmes to more efficiently create and screen for crop varieties to meet the challenges of population growth and climate change. This is not an easy task.

Plants are autotrophic; sessile organisms and their productivity completely depends on the temperature, light levels, and the availability of inorganic substances in the soil. Terrestrial plants are further, and primarily, limited by the availability of water, as the absorption of CO₂ from the air requires water: A few hundred water molecules are lost for each CO₂ molecule absorbed. Therefore, understanding the mechanisms that maintain water balance is critical for optimizing crop growth and fruit production in any given environment.

Productivity-vulnerability trade-offs

Most environmental conditions such as solar radiation, temperature, relative humidity, the CO₂ level in the atmosphere and the quantity and quality of water in the soil directly affect the plant’s biochemical and physiological activity and determine its short-term activity and long-term survival. Plants continuously sense environmental

conditions and dynamically adapt their metabolism and physiology to maximize their productivity and minimize their risk at any given moment. One of the most important and most studied mechanisms for this dynamic optimization is the regulation of the stomatas, the pores in the leaf epidermis that balance the absorption of CO₂ for photosynthesis with water transpiration (Gosa *et al.*, 2018).

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Even a plant growing in a field under well-watered and well-fertilized conditions cannot maintain maximal activity throughout the day, given changes in light intensity, temperature, and relative humidity. The plant’s productive activity peaks during the optimal combination of environmental signals, which will only happen during some parts of the day. It is also likely that the daily optimum will not be the same for different plants in different parts of the same field and on different days (known as the spatial and temporal variability), as it depends on their genotype–environment interactions (G × E). Even two adjacent leaflets of the same leaf will exhibit different levels of activity when they are exposed to different ambient conditions. This high-

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resolution spatial sensitivity is enabled by the guard cells, each equipped with a full environmental sensory system, that control the stomata's activity and hence the flow of water and CO₂.

While photosynthesis, the most basic production process, is fairly consistent throughout the Plant Kingdom and largely similar between algae and higher plants, the mechanisms that regulate the plant's water balance evolved only in terrestrial plants when they colonized the land. In the early vascular plants, such as moss, the mechanism is very conservative, with a high sensitivity to the evaporative demand of the atmosphere, namely the water potential differences between the atmosphere and the leaf (vapor pressure deficit, VPD). Thus, even a slight dryness of the air triggers a rapid closure of the stomata to maintain the relative high water content of the plant. This passive-hydraulic sensitivity or so-called homoiohydric behavior is very effective as a protective trait for maintaining water balance, but it comes at the cost of less CO₂ absorption and lower productivity.

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The adaptation of terrestrial plants to new and more arid environments involved the evolution of an alternative, chemical-hydraulic mechanism based on the level of the phytohormone abscisic acid (ABA) that is produced in the guard cells in response to water stress. It reduces the stomatal sensitivity threshold for VPD such that the stomata are open longer and allows the plant to be productive for a longer period of time. However, the chemical-hydraulic mechanism comes at the expense of increased vulnerability to water stress (Richards, 2000).

The ABA-dependent stomatal regulation created also further evolutionary pressure on vascular plants to evolve a variety of adaptations to conserve water. This ranges from very conservative, isohydric behavior characterized by rapid closure of stomata and more stable water potential to anisohydric behavior characterized by more open

stomata in response to environmental changes and increased risk of water stress. Therefore, different plants have different sensitivity thresholds to similar environmental signals, in general, and stress conditions, in particular.

The classic breeding process aims to maximize a crop plant's yield. Together with agronomic/management processes, such as fertilization, irrigation or pesticide, and herbicide use, breeding enormously increased the productive capacity of crops in recent decades. In fact, FAO reports that the global average yields of the most important cereals—wheat, maize, and rice—have increased by 180%, 150% and 120% over the past five decades (FAO, 2017). However, most of this crop improvement has not been due to improved photosynthesis and metabolic efficiency, but rather a general increase in photosynthetic activity resulting from increased leaf area, greater stomatal aperture and/or stomata remaining open for longer periods (Skirycz *et al*, 2011). It has also been argued that many crop plants are anisohydric; they are less sensitive to or produce less ABA in response to stress, which lowers their sensitivity to water loss and puts them at a greater risk of desiccation. This has created the productivity-vulnerability dilemma: More productive cultivars are potentially more vulnerable to abiotic stress, owing to their faster growth, greater biomass, and slower stomatal-closure response (Fig 1).

Crop improvement under abiotic-stress conditions

Exploitation of the full yield potential of crop plants depends in large part on the time of the growing season during which the plant has optimal growth conditions. Any deviation from these can be considered as “stress” and reduce the plant's productivity. The yield obtained at the end of the season is therefore the total sum of activity minus the “penalties” accumulated during the season. The relative vulnerability to abiotic stress is in fact one of the most significant factors for reduced agricultural yield, as it forces the plant to engage in defensive activity instead of productive activity. In this context, crop yield is not always the biomass of the whole plant, but the mass of its fruits and seeds, that is, the parts that are generated during the plant's reproductive phase and that are harvested and consumed. Thus,

breeding programmes to increase yield pursue a different goal than programmes that merely aim to increase biomass.

Among the many different environmental factors, lack of water in the rhizosphere is the major limiting factor for productivity (Nemhauser *et al*, 2006). Next comes ambient temperature, which affects the plant's biochemical activity both directly and indirectly by changing the relative humidity in the air and thus the VPD, followed by light intensity, the concentrations of nitrogen, phosphate, sulfur, and essential trace elements in the soil and the CO₂ concentration in the air, although the influences of those latter factors will not always be in that order.

The installation of irrigation systems is a possible solution for preventing drought stress and maintaining productivity. However, it is limited by the availability of water. The FAO estimates that 80% of the world's croplands are rain-fed and that irrigation could be increased to maximally cover 6.87% of these by 2050; the remainder will still have to rely on rain as the main source of water (FAO, 2017). Thus, there is a crucial need to develop crops with better tolerance to abiotic stress in general and to water stress in particular.

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The gap between data and practice

Basic and applied plant research have focused much efforts on improving plants' ability to cope with different types of abiotic stress and increasing their productivity. But despite the rich body of genetic, molecular, and physiological information available to researchers, relatively few applications are available to farmers (Dalal *et al*, 2017). This low success rate is not caused by any shortage of research in the field; on the contrary, thousands of articles on the subject are published every year and their number is increasing exponentially. One of the main reasons for the slow translation of basic research into new crop varieties is the great complexity of the problem that involves technical, biological and dynamic environmental

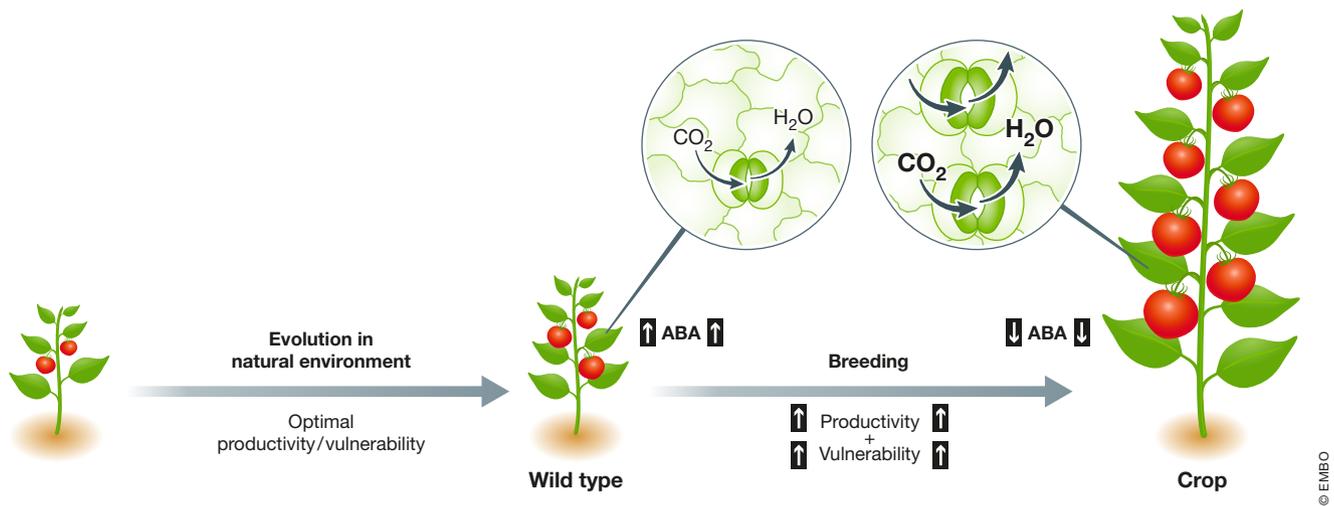


Figure 1. Interactions between productivity and vulnerability of wild plants and crops.

Wild (WT) plants have adapted to environmental pressures to find an equilibrium of vulnerability-productivity trade-offs, which maximizes their adaptation to their natural environment. In contrast, breeding pressure, under non-stress conditions, has maximized yield due to an increase plant biomass, higher numbers of stomata, wider stomatal apertures and stomata that are open for longer periods of time, all of which increase CO_2 absorption. Therefore, breeding pressure has shifted the balance toward increasing productivity under optimal conditions at the expense of increased vulnerability and greater susceptibility to stress.

factors. In practice, the plant's complex responses to various combinations of environmental conditions are difficult to define accurately, difficult to apply comparatively and difficult to replicate. Other problems are the lack of clear standards for accurately defining stress level experienced by the plant, and the use of indirect and ambiguous parameters to measure stress with no reference to any direct physiological measurements. For example, in some drought experiments, there is no reference to or only partial reporting of critical environmental parameters such as VPD, one of the most important atmospheric factors influencing the plant transpiration rate. Finally, there is a lack of a common terminology to describe plant responses to different types of stress (Fig 2). All of these make it difficult to repeat, compare, and draw conclusions from stress studies.

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In many basic research studies, researchers try to overcome some of these difficulties by using non-agronomic model plants under controlled and fixed conditions that do not reflect the variability and uncertainty of the agricultural environment. More than 80% of the plant stress-response studies published in the past three decades were non-agronomic experiments that focused mainly on plant survival rather than productivity (Halperin *et al.*, 2017). One of the most common examples reports drought-resistant plants that exhibit reduced transpiration and remain green for a longer period. Yet, these plants remain green simply because they survive the stress by reducing their productivity. It is not at all certain that they exhibit any agronomic resistance, namely no yield penalty relative to irrigated control (Violet-Chabrand & Lawson, 2019).

From the laboratory to the field

In contrast, breeding programs have for long aimed to reduce the vulnerability of elite crops while maintaining high levels of productivity. The initial step in a breeding program (pre-breeding) is to define the problem, characterize the desired traits and find such traits in the germplasm or gene resources. If the aim is to improve biotic

resistance, the definition of the problem is usually straightforward—for instance, a plant pathogen—the definition of resistance traits is obvious—the plant does not develop disease symptoms and its yield is similar to untreated control plants—and new varieties can be tested relatively easy.

The improvement of abiotic-stress responses, though, and drought responses in particular, is more challenging as the stress definition is dynamic and there are numerous genes involved, resulting in highly complex traits and dynamic $G \times E$ interactions. To start with, the stress phytohormone ABA has an enormous effect on gene regulation, changing the expression profile of thousands of genes (Dalal *et al.*, 2020). The accurate definition of the stress conditions and the desired traits is therefore an even more important step for pre-breeding. Nevertheless, many programs fail to meet that requirement.

For example, a pre-breeding program that aims to improve crop yield under drought conditions might define rapid stomatal closure or a deeper root system as goals to increase soil water availability, that is, as drought-beneficial traits. Yet, these traits are only avoidance mechanisms that limit or prevent the plant from reaching and having to cope with drought stress. These would be better suitable for breeding programs that

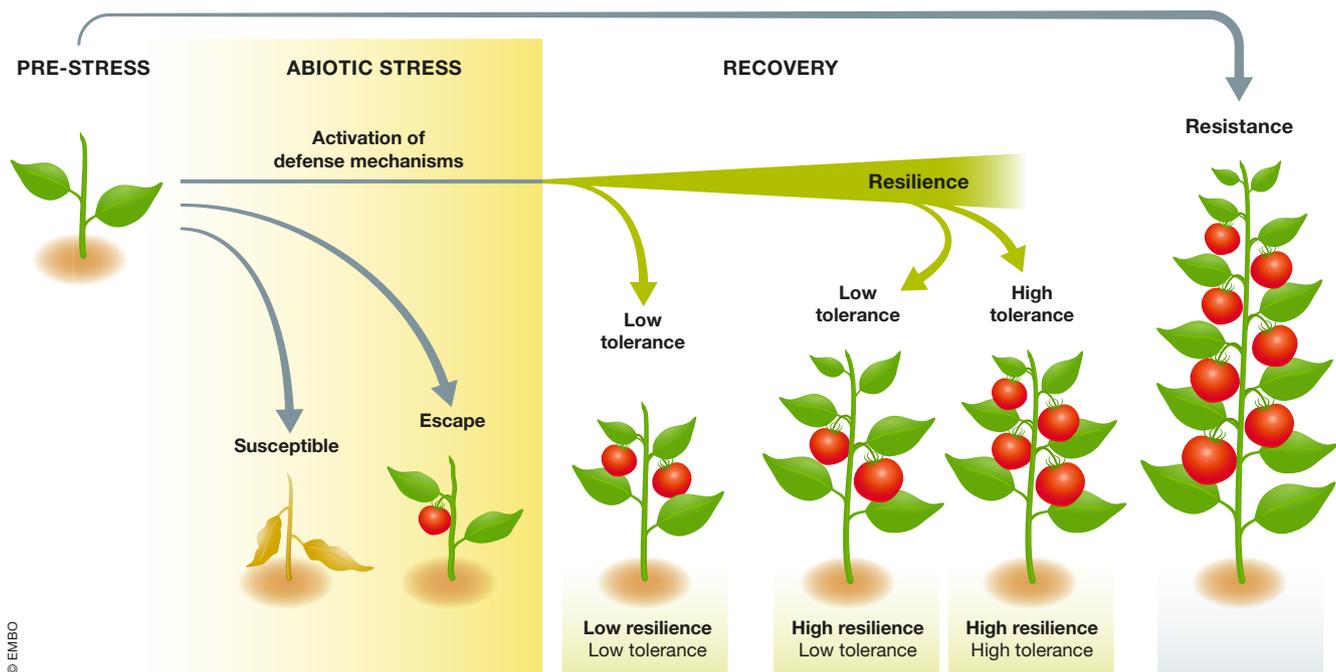


Figure 2. Terminology of crop-plant stress responses.

When a plant is exposed to stress conditions, it activates molecular, anatomical, physiological, and biochemical defense mechanisms that allow it to cope with stress—for instance growth inhibition, stomatal-conductance reduction, suberization of roots' cell walls, etc.—and engage in damage control, such as anti-ROS activity and osmolyte accumulation. The plant's response to the abiotic stress can be characterized in terms of behavior. If the plant is unable to cope with the stress and sustains damage from which it cannot recover, it is **susceptible**. A plant that enters its reproductive stage early and ends its life cycle without fulfilling its full genetic potential is employing an **escape** response. A **recovery** phase occurs if the stress and/or when the defense/adaptation mechanisms enable the plant to increase its vegetative activity. The **resilience** of a plant is a measure of the rate of its return to optimal vegetative performance, which will allow it to fulfill its reproductive potential. A plant with lower resilience exhibits a slower rate of recovery, revealing a **low resilience-low tolerance** phenotype. If the plant shows good resilience, but sustained damage in the reproductive stages, the plant will be relatively large, but with low yield and its stress response will be defined as **high resilience-low tolerance**. If the defense mechanisms are effective, a complete vegetative recovery and minimal damage to the reproductive and yield stages are expected and the plant's behavior will be defined as **high resilience-high tolerance**. Alternatively, if the plant is completely immune to the stress and overcomes it without sustaining any damage (compared with non-stress control), it is **resistant**. Halophyte plants that are not affected by high concentrations of salt are an example of resistance to abiotic stress.

aim to increase the plant's survivability even at the expense of yield production, for instance garden flowers or golf-course turf. Similarly, defining an earlier flowering time as goal, so that the crop life cycle will end before drought conditions build up (escape mechanism, Fig 2) does not meet the definition of productivity improvement, even if it is helpful for developing crops adapted to a semi-arid climate with a short rain season or areas characterized by long, cold winters and short growing seasons.

Another main challenge is to define the stress conditions and the stress-threshold level. Technically, the threshold for drought stress is the point at which the amount of water in the rhizosphere (θ) limits transpiration (θ_{crit}). This threshold point depends mainly on the transpiration capacity and its regulation by the plant; different plants will therefore have different θ_{crit} points

depending on their physiological, anatomical and biochemical properties, as well as their sensitivity to ambient atmospheric conditions (Bänziger *et al*, 2000). Practically speaking, the drought-stress level must be experimentally determined based on the plants' physiological response profile and in relation to an well-watered control (Fig 3).

Functional characterization of the plant responses to the environment

Creating a physiological behavioral profile of the plant-water-environment relationship of each candidate line under conditions that mimic the expected environmental conditions is also important for achieving the pre-breeding goals. For example, canopy stomatal-conductance kinetics are an important trait for yield prediction. One of the greatest challenges for breeders is to

quantitatively characterize these functional traits from an early growth stage and during the course of the growing season.

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The use of image-based, high-throughput phenotyping systems to solve the $G \times E$ dynamic challenge has had only limited success during the past decade, as morphological changes appear relatively late in the response to stress, compared with more rapid physiological changes. For example,

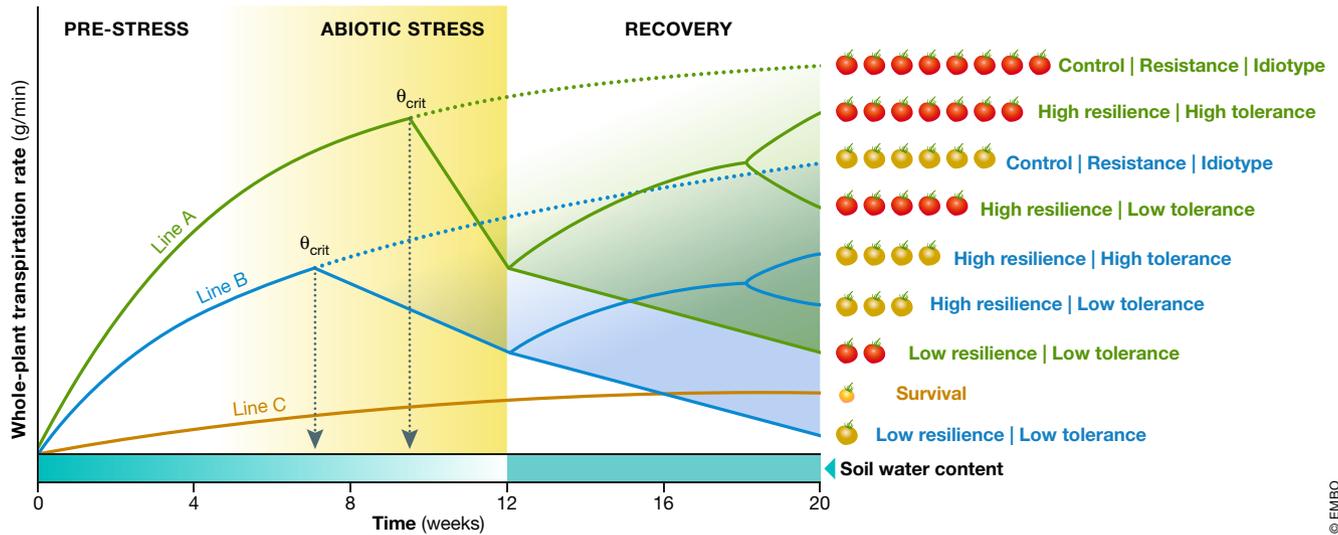


Figure 3. Comparative functional characterization of crop plants' responses to seasonal drought (functional $G \times E$ phenotyping).

Figure 3 describes a comparative physiological screen that examines relative differences in plants' responses to the environment; for example, whole-plant transpiration-rate kinetics. This functional characterization examines and compares the lines under the expected environmental conditions and at each growth stage. The figure shows three stages of "exposure to seasonal drought" of three different lines; Line A (green) has a higher yield potential than Line B (blue) and Line C (orange). Plants of all lines were monitored simultaneously under similar environmental conditions. The scenario begins with optimal growth conditions, pre-stress, during which the functional parameters are at their best and bring Line A to faster growth (and transpiration) than Line B and C. Later in the scenario, there is a gradual decrease in the soil water content (drought stress). However, the actual physiological stress phase does not begin at the moment that the irrigation is turned off (Week 4), but rather when the amount of water in the soil becomes limiting for plant transpiration: θ_{crit} . At this point, the plants will respond different from that of the control plants under optimal conditions (dashed line). Lines A and B have different θ_{crit} points (indicated by the arrows); that is, they have different stomatal-conductance sensitivity thresholds and/or different root performance. The low-transpiring Line C did not reach its θ_{crit} , yet will produce a relatively low yield due to its low productive capabilities, which are characteristic of the type of survival behavior it represents. From the θ_{crit} point onward, a functional difference develops between the treated plant and its irrigated control. The larger the difference, the greater the expected yield penalty at the end of the season (reflected in the figure as a fading background). An optimal breeding goal would be the identification of an idiotypic plant that will not show any difference in functional parameters relative to a well-watered plant-resistance mechanism. The third stage in the scenario is the recovery stage (Week 12) during which irrigation is resumed. In practice, measurement of the recovery rate allows a better comparison of the stress-adaptation mechanisms. The more efficient these mechanisms, the faster the plant will recover. In addition to the above indices that measure the vegetative (productive) phase, it is also important to examine the reproductive phase (e.g., the number of flowers, fruit-setting) and especially yield, in order to construct a full stress-response profile. It is important to note that a much shorter response profile characterization could be effectively conducted in order to shorten the screening process.

nitrogen shortage will reduce transpiration within a few hours while visible yellowing will take a few days to appear. Moreover, the number of $G \times E$ combinations that provide an optimal match between the environmental conditions and the biochemical and physiological state of the plant are so numerous that only a continuous and comparative functional test can create a representative behavioral profile of the plant and predict its behavior in the field. New, high-throughput, functional-phenotyping platforms that enable continuous monitoring of plant-water relations of many lines simultaneously while automatically controlling the applied stress conditions for each plant have shown better success in addressing this $G \times E$ challenge (Fig 3) and providing breeders with quantitative characterizations of complex traits. Integrating this type of physiological-functional characterization into breeding programs will improve

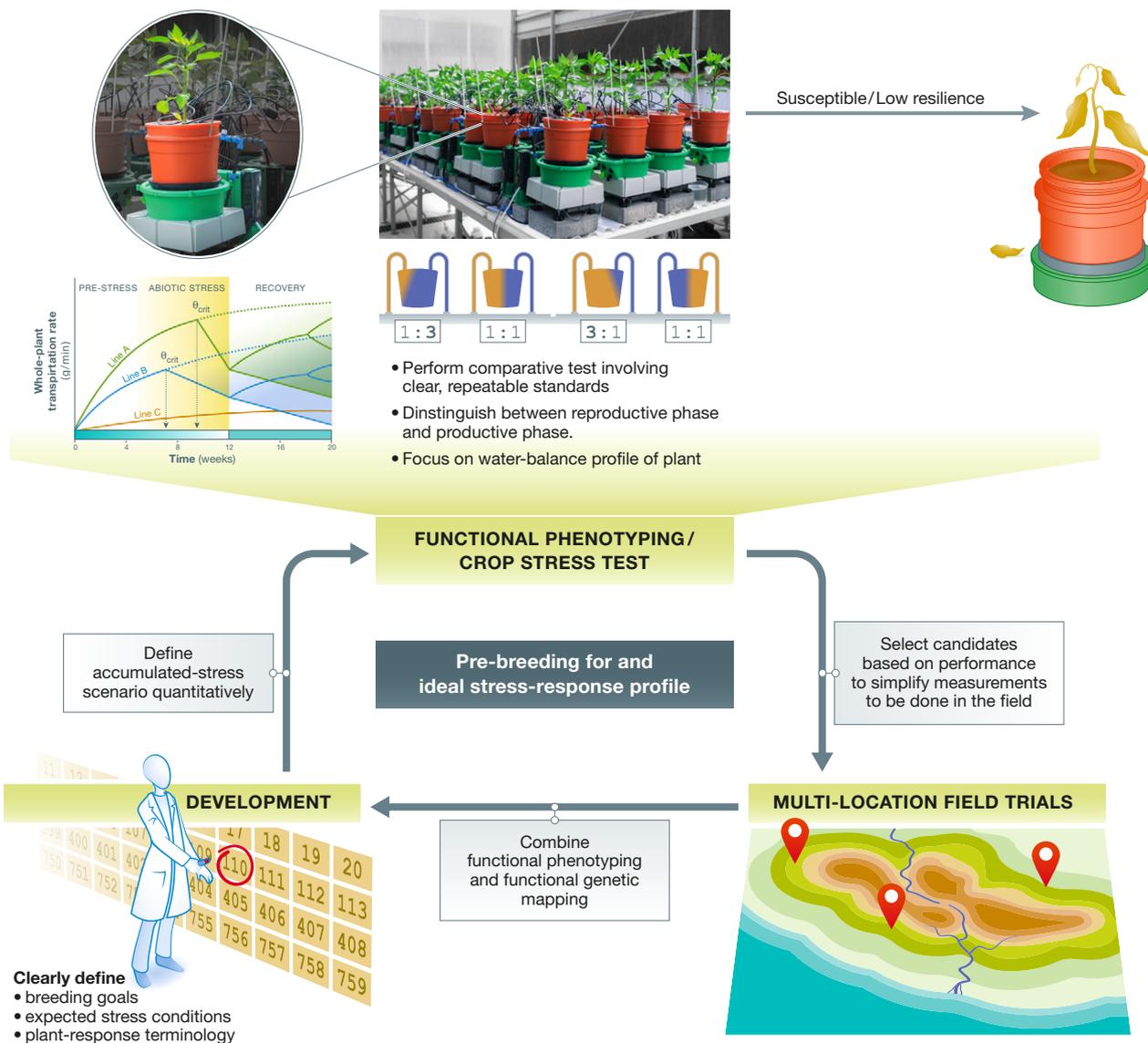
the evaluation and selection of parental lines and/or gene discovery and shorten the amount of time it takes to get promising candidates into the field (Fig 4).

Finally, an important point in the functional characterization of plant responses is the distinction between the vegetative stage during which a plants grows and accumulates biomass and its reproductive stage during which flowers and fruits develop. Good vegetative development is indeed the basis for yield production, as bigger and healthier plants can produce more fruits and seeds. Yet, if stress injures the reproductive organs, it will likely cause a yield penalty, regardless of the plant's performance during the vegetative stage. Thus, breeding programs should treat the vegetative growth and the reproductive growth as distinct breeding goals. For example, corn (*Zea mays*) is an isohydric C4 plant that has a high resilience against water stress but a low

reproductive tolerance. That is, if a corn plant reaches a sub θ_{crit} during the flowering period, it may lose a significant percentage of its yield, mainly due to the susceptibility of the female flower to stress and despite the plant's overall ability to cope with the stress (Figs 2 and 3). This may explain why some of the properties found in recent corn breeding programmes to improve yield under drought are related to shortening the anthesis-silking interval and increasing the number of ears per plant (Bänziger *et al*, 2000).

The goal: finding the idiotypic plant performances

As part of the $G \times E$ characterization, many breeding programs use multi-location field trails in which the same lines are tested under different environmental conditions. Therefore, the genotype selection is based on plant



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Figure 4. Optimization triangle for pre-breeding.

Figure 4 depicts a scheme for identifying, at the pre-breeding stage, complex functional properties with the potential to improve the plant's ability to cope with stress. The development phase requires clear definitions of the environmental conditions of interest and the desired behavioral characteristics at each of the plant's growth stages. The initial selection of candidates from the seed bank will be made according to the existing knowledge and program goals. After that, the stress scenario should be defined quantitatively and compared with the appropriate control treatment. The desired candidates should be tested in a high-throughput functional-phenotyping system that incorporates feedback control of the stress level for each plant in the array and allows the rapid screening of many candidates simultaneously. [The photograph in the figure shows the PlantArray functional screening platform in the process of screening a plant's response to drought. This system is located at the Israeli Center of Research Excellence (ICORE) for Plant Adaptation to the Changing Environment, at The Hebrew University of Jerusalem.] The relatively quick physiological profiles derived from the functional test can be used to disqualify unsuitable candidates.

performance under diverse environmental conditions. This method is relatively expensive, and the success rates of the multi-location approach are relatively low because it requires contradictory capabilities of the genotype; on the one hand, very high levels of plasticity for many physiological properties and, on the other, consistently high yield.

An analogy for this contradiction is a heavy, off-road car that can also win a Formula 1 race. Finding the ideotypic line takes a lot of time and resources, since there are many potential candidates. Integrating functional $G \times E$ characterization into the early development stages of a pre-breeding program may improve the germplasm selection and

thus reduce the number of candidates with the potential to do well in the field (Fig 4).

To date, only a few drought-tolerant genotypes have been released from publicly funded programs such as the International Maize and Improvement Center (CIMMYT) or the International Rice Research Institute (IRRI), or from private seed companies. This reflects the

complexity of the abiotic-stress response mechanisms and the difficulties of implementing agronomic tolerance and economically valid solutions. Finding the off-road Formula 1 vehicle—crops that are less sensitive to water stress and produce even higher yield—is a formidable puzzle to solve until 2050.

Conflict of interest

The author declares that he has no conflict of interest.

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