

Creating a paradigm shift in plant breeding and plant phenotyping

Innovative plant breeding strategies accessing the beneficial relationships between soil microbes and plants could help develop varieties that are more resilient to climate change and soil conditions. Dr Omirou and Dr Fasoula at the Agricultural Research Institute, Cyprus, describe how moving away from conventional multi-plant, densely-grown field plots, and using innovative selection designs fitted to individual plants grown at ultra-wide distances, overcomes several barriers to effective plant phenotyping. Their experimental planting patterns, called Honeycomb Selection Designs, when combined with prognostic breeding, can evaluate plant productivity and trait performance stability in the same generation, and can help select for advantageous plant-microbiome associations.

Plant breeding relies on the evaluation of field performance followed by the selection of desirable plant phenotypes (observable and measurable characteristics or traits). The same plant genotype will produce different phenotypes, depending on the environment in which it grows. Since a plant can't move, a significant part of its environment is the specific soil spot where it grows, which may differ from soil only a few metres away. This soil heterogeneity involves various factors, including the distribution of the soil microbiome. Plants acquire many desirable traits through their interaction with soil microbial communities, an effect which could be put to beneficial use.

Now, a new trend in plant breeding is being directed at harnessing the soil microbiome — the community of bacteria, archaea, viruses, fungi and protozoa present in the soil — to develop crop varieties with improved abilities to benefit from the interaction of their roots with the associated microbiome. Soil microbial assemblages can, for example, suppress plant pathogens or enhance nutrient uptake and response to stresses like drought and salinity. By improving the interaction between plants and soil microbiomes agricultural scientists could mitigate and even reverse negative effects of climate change and of soil degradation on crop yields.

The soil microbes associated with plants can be endophytic (living within a plant) or rhizospheric (present around the plant root system). Examples of beneficial associations are rhizobial

bacteria supporting nitrogen fixation in legumes and arbuscular mycorrhizal fungi (AMF) enhancing drought tolerance. Breeding for enhanced nitrogen-fixing ability in legumes, for example, could help reduce the costly application of nitrogenous fertilizers. The innovative plant breeding strategies adopted by Dr Omirou and Dr Fasoula seek to exploit the inherent properties of microbiome complexity to create more resilient varieties.

ADVANTAGES OF HONEYCOMB SELECTION DESIGNS AND PROGNOSTIC BREEDING

In plant breeding programmes, efficient selection of desired crop traits depends on determining an appropriate unit of plant phenotyping in the field. Conventional plant breeding uses multi-plant, densely grown field plots as the units of plant evaluation and selection. However, this practice can limit accurate phenotyping of plants and their associated root systems. It also cannot take the best advantage of current advances in genomic information. To address some of the problems with conventional methods, Dr Omirou and Dr Fasoula use innovative selection designs where individual plants are grown at ultra-wide distances to fully express their genetic potential and maximise phenotypic expression and differentiation.

These Honeycomb Selection Designs place individual plants in triangular grid patterns (hence 'honeycomb') at distances wide enough to exclude the plant-to-plant interference (competition) found in traditional selection plots.

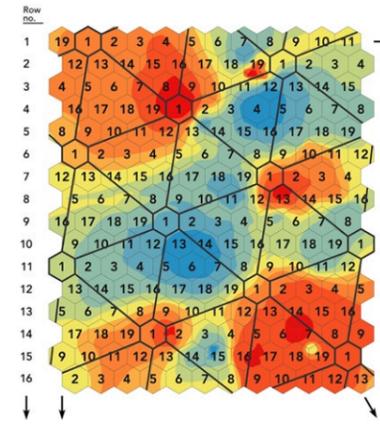


Figure 1. The replicated-19 Honeycomb Design is one of the many constructible Honeycomb Selection Designs and evaluates plants of 19 lines or genotypes. The numbers represent the position of plants in the field and the hexagons represent the area exploited by each plant. The plant-to-plant distance is 1m (as seen in actual field photo, overleaf). The design is depicted on top of a multicolour background that signifies the microbial heterogeneity of the whole field. Each colour corresponds to different relative abundance of microbial genera. Plants of each line are arranged in an ascending numerical order and are placed in the corners of moving equilateral triangular grids, exemplified here for line 1. The grids spread across the field and sample effectively for spatial heterogeneity. Moving grids ensure reliable measurement of the trait stability of each line through the use of the stability (or homeostasis) index, $SI = (\bar{x}/s)^2$, where \bar{x} and s are the mean yield and standard deviation of the plants allocated across the triangular grid of each line.

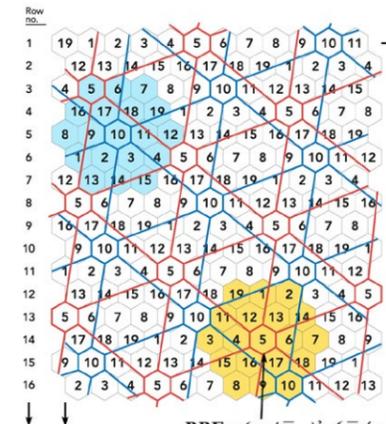


Figure 2. Additional properties of the replicated-19 Honeycomb Design are highlighted. The same properties apply to all Honeycomb Selection Designs. In every field position, each plant occupies the centre of a moving and complete replicate, as shown for two random plants of lines no. 10 and no. 5. Moving replicates (or moving rings) are illustrated with blue and orange shades and include representative plants of all 19 lines. The use of the plant yield index $PYI = (x/\bar{x})^2$ reduces the masking effect of spatial heterogeneity on single-plant yields because x is the yield of each plant and \bar{x} is the mean yield of the surrounding moving-replicate plants. The product of PYI and SI is a number unique for each plant and is the value of its phenotyping equation PPE under the specific conditions.

Soil heterogeneity includes a variety of factors, like availability of plant nutrients, soil structure and pH, ability to hold water and importantly, the presence of pathogenic or beneficial microorganisms living in the soil. The

Competition for resources in densely-grown plots prevents individual plants from maximising their phenotypic expression and realising their full genetic potential. This has not previously been properly accounted for and explains why breeding for increased yield has progressed rather slowly. Competition can create large variations in plant yields and other traits of interest and introduce bias, since a breeder will not know if this is true genotypic superiority due to some plants gaining advantages at the expense of others nearby.

The Honeycomb Selection Designs overcome the effects of spatial variation in soil characteristics by the formation of moving, complete replicates and triangular grids extending across the whole field (Figures 1 and 2).

Plants acquire many desirable traits through their interaction with soil microbial communities, an effect which could be put to beneficial use.

designs enable effective sampling of the full spectrum of soil and environmental heterogeneity, ensuring that all plants and sibling lines are allocated under comparable growing conditions in both fertile and less fertile spots. The triangular grid shown in Figure 1 illustrates how the plants of line no. 1 are allocated effectively across different soil conditions and spatial variation.

Conventional plant breeding systems measure productivity and performance

stability by comparison with the next generation. A methodology called prognostic breeding enables phenotyping for high and stable yield of plants and sibling lines grown in the Honeycomb Selection Designs. Two phenotyping equations evaluate yield and stability of performance in the same, rather than in successive generations. This improves selection efficiency, reduces selection cost, shortens the time to release of cultivars and accelerates the annual genetic improvement possible. The main plant phenotyping equation (PPE) considers the yield or other traits of each individual plant in comparison to its neighbouring moving ring plants, factoring in how stably the sibling line expresses the trait under consideration (shown in Figure 2).

During the trial, each plant obtains a unique identification number based on their equation value. This number is very informative during the process of phenotyping; it allows quick comparisons of multiple experiments across years and locations. The application of the equations leads to the development of density-neutral varieties (that perform well under a broader range of plant densities) and can better withstand the effects of climate change and water scarcity. This is because selection based on the plant yield index leads to good performance even at the lower plant density limit. For example, this can happen during a drought when only a percentage of crop plants in the field survive. Selection based on the

stability (or homeostasis) index secures good performance under high planting densities, as is the situation when the crop growing conditions are optimal and all plants survive.

In a recent paper, Fasoula and colleagues outlined how these alternative approaches help to overcome the various barriers in plant phenotyping. As discussed, barriers to selection efficiency include the effects of interplant competition and spatial heterogeneity. Also, the limited

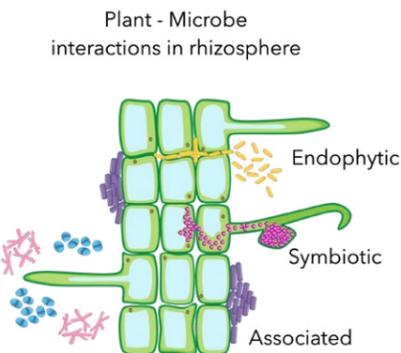




Photo shows individual wheat plants grown in a replicated Honeycomb Selection Design in the field.

seed supplies in early segregating generations following a cross are not an issue in Honeycomb Selection Designs since they evaluate single plants. This permits the effective valorization of Plant Genetic Resources and Crop Wild Relatives for which seed supplies are usually limited. Finally, the new methodology avoids the restrictions of current statistical approaches.

These Honeycomb Selection Designs can be used in all plants amenable to breeding, regardless of their mode of propagation and annual or perennial habit. The designs facilitate the possibility of mechanising and automating selection for yield and

is the greatest limiting factor on yield and will become even less predictable with climate change. This crop is known to be associated with arbuscular mycorrhizal fungi (AMF), which have been found to enhance drought tolerance of the host chickpea plant by altering the host hormone balance or enhancing the movement of water from roots to shoots.

Omirou, Fasoula and collaborators are studying the effect of water limitation on plant growth and the AMF biome in chickpea genotypes previously selected in the field using the Honeycomb Selection Designs. To further investigate specific

Honeycomb Selection Designs and the phenotyping equations of prognostic breeding can be used in all plants and categories of crops.

other important plant traits. Finally, and of particular interest here, their method allows more extensive root growth and permits selection of plants with improved root architecture, efficient in capturing resources by roots and exploiting microorganisms that can promote plant growth. This practice can facilitate ongoing global efforts for overcoming the challenges of root phenotyping in the field. The methodology is also applicable to Organic Agriculture.

THE EXAMPLE OF CHICKPEA

Chickpea is one of the most important legumes and is grown in arid and semi-arid areas where water availability

details, they chose two genotypes for controlled pot trials. Their recent report describes the response of the two chickpea genotypes grown under well-watered and limited water conditions to see if water limitation affected nutrient accumulation and mycorrhizal colonisation. The responses of the AMF assemblages associated with the plant root systems were also evaluated.

One of the chickpea genotypes showed no difference in growth or AMF colonisation when water was limited. For the other genotype, drought stress reduced growth and this was also associated with suppressed

AMF colonisation. The results suggest that chickpea genotypes can differ in their relationships with AMF. Varieties of chickpea could therefore be bred for improved drought tolerance and enhanced AMF association. The above selection methods along with availability of beneficial microbiomes will successfully support such an endeavour.

DEVELOPING NATIVE MICROBIAL SOIL GENETIC RESOURCES

Selecting for plant phenotypes that attract microorganisms able to promote plant growth could provide an effective strategy for tackling the adverse conditions of climate change. Identifying the core microbiome of plant species suitable for breeding, isolating the microbes and applying appropriate inocula could also help create field conditions that favour plant performance. Microbial inocula derived from relevant agricultural ecosystems will ensure the closest possible relationship between effective microbial guilds and crop plants.

Metagenomics (the study of genetic material recovered directly from environmental samples) is expected to provide **advanced microbial solutions for future more sustainable agricultural practices**. The researchers' group leads a state-of-the-art environmental microbiology and biotechnology unit (<https://magnet.moa.gov.cy/>) for studying the native microbial diversity of Cyprus. Base inventories of the microbiomes are also being undertaken to monitor changes in soil biodiversity over time in the main agricultural areas of the country.

In addition, Dr Omirou and Dr Fasoula are working to isolate and characterise soil microbes that promote plant growth under drought, through an international project funded by the EU (<https://sites.unica.it/supreme/>). The aim is to combat soil impoverishment and to reduce the use of water, fertilizers and pesticides by harnessing the microbiome potential to stabilise soil and promote plant growth under adverse conditions. The hope is that this will eventually benefit vulnerable agricultural communities living in semi-arid and arid areas in the Mediterranean.

Behind the Research



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Research Objectives

Drs Omirou and Fasoula are creating a national infrastructure of soil genetic resources and developing more resilient, density-neutral varieties which effectively exploit plant-microbe associations.

Detail

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Personal Response

In your opinion, what is the scale of soil and microbiome degradation in agriculture and how challenging will it be to re-establish healthy plant-microbiome associations?

Soil is the cornerstone of food production and is in danger due to the overuse of chemical inputs and climate change and of course the long term monocultures and monocropping that are still dominating farming practices globally. The rate of soil degradation in arid and semi-arid regions is very high and threatens the productivity of agro-ecosystems.

The challenge for scientists and policy makers is to adopt novel concepts that are able to sustain and improve soil productivity. The development of microbial tools, along with the development of resilient, density-neutral (or density-independent) varieties that can effectively exploit and thrive even under limited growth resources without requiring higher planting densities for optimal productivity, is an integral part of this effort and could be enormously useful for a climate-changing agriculture.