



Closing the yield gap: can metabolomics be of help?

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The difference between the potential yield of a crop and the yield obtained by farmers—often called the yield gap—is explained by environmental cues and/or deficient management practises. Up to now, it has been mostly addressed by mitigating stresses, i.e. by improving management practises, and little by the development of more resilient varieties. However, increasing the inputs in order to tackle the yield gap is getting less and less sustainable in the context of more frequent and stronger climate events, a growing population, and increasingly limited land and input availabilities. Thus, the aim should be for a more sustainable biomass production, which involves, among other strategies, the breeding of resilient crop varieties. Melandri *et al.* (2019) investigated the leaf metabolic parameters associated with yield loss of 292 rice varieties submitted to drought stress in the field.

They conclude that only the metabolic profiles obtained under drought stress are good predictors of yield loss, suggesting that metabolism is a major target to be considered for breeding drought tolerant varieties. They concluded that ‘photorespiration, protein degradation and nitrogen recycling were the main processes involved in the drought-induced leaf metabolic reprogramming’. Interestingly, a large number of amino acids that accumulated under drought stress, including proline, appeared to positively correlate with yield loss, together with malondialdehyde, a product of lipid oxidation, suggesting that amino acid accumulation might be more a symptom of drought stress than a mechanism to mitigate it. In contrast, the activity of the enzyme dehydroascorbate reductase was strongly negatively correlated with yield loss, suggesting a central role of the ascorbate–glutathione cycle in drought tolerance. Such metabolomic study, allowing to identify biological pathways involved in stress resilience in crops grown in the field, when combined with other phenotyping and genomic tools, could allow for precision breeding towards the fast development of stress tolerant crop varieties.

Despite our knowledge that environmental conditions have a major effect on crop yield, and that as a result of climate change the stresses encountered by our crops in the field are

increasing in frequency and intensity, a large part of breeding efforts is still targeting yield gain under optimal field growth conditions, which often require large inputs. Such strategy has allowed for the green revolution but will be less and less sustainable in the future because access to inputs, such as water and fertilisers, will be less and less affordable, including in western countries.

Environment has a very strong influence on the phenotype and yield of plants. Even very small variation in environmental conditions can lead to dramatic variations in growth and metabolism of the plants, as shown by Massonnet *et al.* (2010) where the authors grew several Arabidopsis accessions in 10 different laboratories under the exact same growth conditions and showed that accessions still showed very large inter-laboratory variations in growth and metabolism. The genotype \times environment, or genotype \times environment \times management interaction factor in the field, is very large, which explains why breeders develop new varieties at different test sites to take it into account (Atlin *et al.*, 2017; Gilliham *et al.*, 2017).

The yield gain for our crops consistently increased over the last century. However, a plateau, or at least a steep decline in the yield gain, has become apparent for most of our major crops (Espe *et al.*, 2018; Hall and Richards, 2013), suggesting that we might not be able to increase our plant-based biomass production enough to feed 9–10 billion humans by 2050. Indeed, Espe *et al.* (2018) show that, when the decline in yield of old rice cultivars over time is taken into account, there has been a marginal yield gain for the last 33 years in rice cultivars cultivated in California. Equally worrying, maize varieties not farmed optimally in the USA, for example due to lack of fertilisation and irrigation, showed very marginal yield gains for the period 1987 to 2015, in sharp contrast to the same varieties grown under high yielding environments where significant yield gains were observed (Assefa *et al.*, 2017). This means that we have been breeding for yield gain under optimal growth conditions, but that the gains disappear under sub-optimal conditions due to yield loss, which unfortunately will be likely more and more frequent due to climate change, and increased scarcity of inputs. Another issue to explain the poor increase in yield gain worldwide is that farmers in developing

countries observe large yield gaps, in part because they rely upon outdated varieties from the 1970s, as efforts to develop novel varieties largely focus on the western market (Atlin *et al.*, 2017), but also largely because their field conditions are sub-optimal and thus require the development of specific resilient crop varieties.

Breeding for resilience has been achieved for species such as grasses (Parsons *et al.*, 2011), demonstrating that breeding for stress tolerance is achievable and can successfully become the aim for breeding of our main staple crops. Plant phenotyping can help for the process, but only if it is achieved together with breeders and goes with a comprehensive description of the key constraining conditions prevalent in the field, thus allowing proper integration of data obtained in the field to large datasets, which is necessary for crop modelling (Araus *et al.*, 2018).

Among the toolkit of techniques comprising plant phenotyping, metabolomics appears to be most powerful for predicting yield (Riedelsheimer *et al.*, 2012a) and thus allows us to reduce the gap between phenotype and genotype as molecular markers or even genes can be associated with metabolites, leading to precision breeding (Riedelsheimer *et al.*, 2012b).

Melandri *et al.* (2019) measured the metabolome and several enzyme activities in flag leaves of rice varieties submitted to field control conditions or 14 days of drought stress at flowering, and then search for metabolites or enzyme activities associated with yield loss. Theirs is one of the very few large-scale metabolomics studies which has been performed under field conditions, and one of the first investigating the metabolic response of crops to drought in field with the aim at linking metabolite profiles to yield loss.

The metabolic profile of rice varieties under drought, but not under well-watered conditions, predict yield loss

Melandri *et al.* (2019) observed that the metabolic correlation network is much stronger under drought stress than under well-watered growth conditions, with, in particular, many amino acids and sugars correlating together, and both groups tending to negatively correlate with organic acids. This suggests that conditions hindering carbon assimilation lead to a tight control of metabolic reactions, associated in this study with an increase in photorespiration and protein catabolism as the levels of many amino acids increased. Thus, because the control of the metabolism is more important under stress conditions in field, metabolomics should be considered for breeding programmes targeting the development of stress tolerant varieties.

In agreement, the metabolic profiles of plants facing drought stress, and not of plants grown under well-watered conditions, appeared to correctly predict yield loss. It again points towards the importance of the leaf primary metabolism in the control of grain development under drought stress, but also demonstrates that metabolism might be less important under optimal conditions, where factors related to, for example, development, harvest index and architecture, might be more important. This result could potentially explain why Assefa *et al.* (2017) observed a clear yield gain in maize varieties cultivated under highly fertile

growth conditions in the USA during 1987 and 2015, but virtually none for the same period when the varieties were grown under sub-optimal conditions. We are not breeding against the yield gap, and to achieve it, we probably need to consider metabolic traits and identify associated molecular markers.

Drought stress leads to the accumulation of amino acids and sugars, but none are predictors for, or negatively correlated with, yield loss

As expected, the levels of a number of so-called compatible solutes such as proline, raffinose and galactinol increased in response to drought. However, unexpectedly, their levels under drought were not good predictors (galactinol, raffinose, proline) and were either not correlated (galactinol, raffinose) or positively correlated (proline) to grain yield loss. This finding goes against the assumption that these compounds protect the plants against drought stress for preserving seed production, and suggests that more research is required to identify the exact role of these compounds in plants growing under stress in field conditions. In line, Mwadingeni *et al.* (2016) did not observe in bread wheat varieties submitted to drought in the field any significant correlation between proline levels and most yield-related traits, the thousand seed weight being even negatively correlated to proline content. These results question the relevance of efforts towards increasing the levels of compatible solutes in crops for stress tolerance and justify the need for field studies where plants usually face several stresses at the same time.

The ascorbate-glutathione cycle, and more generally ROS scavenging mechanisms, might be a target for breeding drought tolerant rice varieties

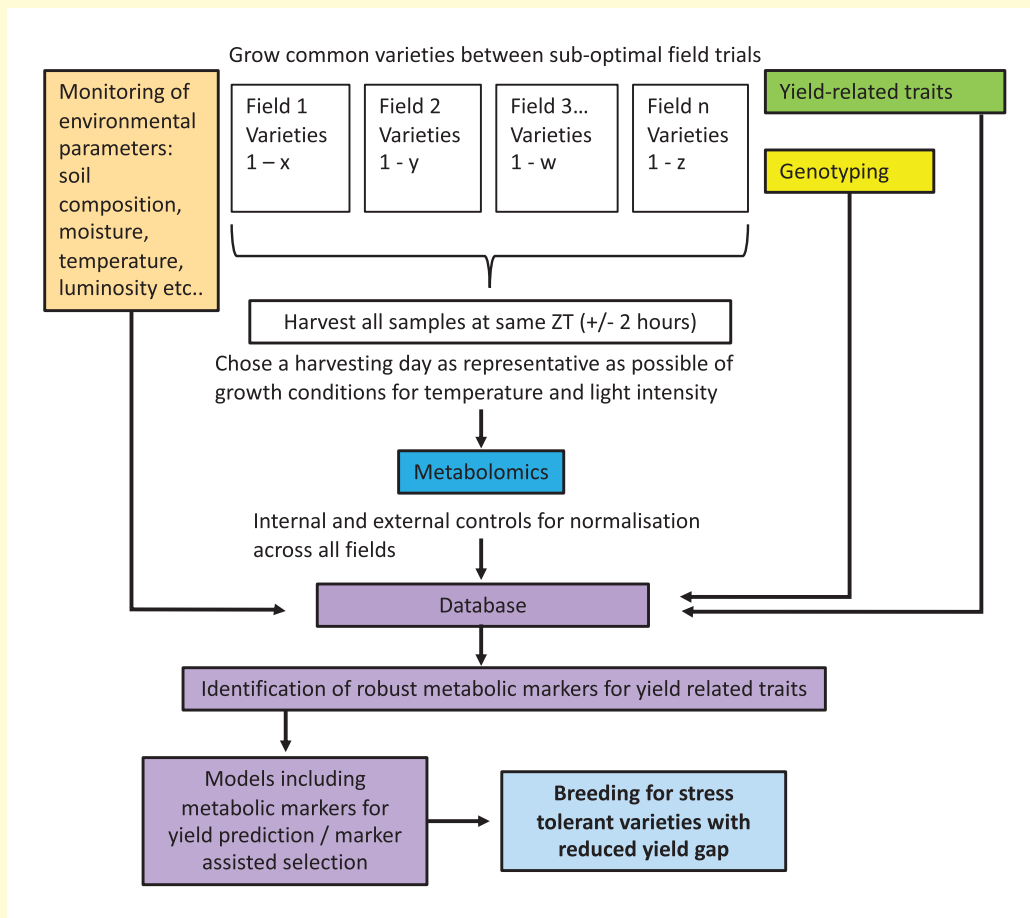
Melandri *et al.* (2019) identified dehydroascorbate reductase as negatively correlated to—and an excellent negative predictor of—yield loss, along with some extent monodehydroascorbate reductase. Also, ascorbate peroxidase and catalase were negatively correlated with the metabolites associated with stress. This highlights the importance of the ascorbate glutathione cycle as an important metabolic pathway for rice resilience to drought (Wei *et al.*, 2015). The ascorbate-glutathione cycle has been studied for many years, leading to numerous papers demonstrating its importance for plant tolerance to abiotic stresses (Yendrek *et al.*, 2015), including a rice RIL population submitted to drought stress (Prakash *et al.*, 2016). Thus, the enzymes involved in ROS scavenging should likely be considered primary targets for the development of drought stress tolerant varieties.

Perspectives

The study by Melandri *et al.* (2019) demonstrates the importance of the metabolism in the regulation of yield under drought stress. The authors confirm previous studies showing that metabolic networks and metabolic predictors for yield do

Box 1. The use of metabolic biomarkers for breeding stress tolerant crop varieties.

Many metabolites have very short turnovers, which explains their diurnal variations, as well as why their contents vary even in response to short term changes in the environment. Thus, their use as biomarkers in field conditions might be an issue as biomarkers identified under one field trial might not be found in another similar trial, thus impairing their use for breeding purposes. In order to circumvent this issue, several measures can be taken. The environmental parameters must be recorded over the course of the trial and in particular during the day when the samples are harvested for metabolomics. To avoid the bias of diurnal variation of the metabolic content, samples for all field trial should be harvested at the same ZT (Zeitgeber time). Also, when possible, weather conditions on the sampling day should represent the general growth conditions the plants encountered in the field; i.e., avoid sampling on a cloudy and cold day if plants have been encountering warm and sunny days in the weeks leading up to the trial. Metabolomics analyses should include the use of internal and external standards for normalisation. Ideally samples from the different trials should be analysed randomly and not sequentially, but, in the case of the development of a large database for the collection of the data, this will not be possible. All data should be entered within a database, which ideally should be published open access in order to allow other groups, including breeders, to retrieve previous data and thus obtain more statistical power for developing crop yield models and identify DNA markers. It is unfortunate that presently a large majority of the data obtained in the crop science area are not stored in common databases, in contrast with what has been achieved for many years for genomic data repositories, for example. We expect that with the aggregation of metabolic data obtained from a large number of field trials and varieties, together with field environmental variables, it will be possible to identify robust metabolic markers, and the associated DNA markers, and thus develop an efficient and fast breeding programme targeting the reduction of the yield gap.



vary with environmental variations, and importantly, suggest that metabolism is a major factor controlling yield loss under drought field conditions, in contrast with optimal growth conditions where the primary metabolism might be less important for the control of grain yield. Thus, crop breeding efforts for drought stress tolerance should differ in the traits they assess compared to breeding under optimal growth conditions, and, particularly, they should include the assessment of metabolic parameters in order to identify metabolic molecular markers associated with drought tolerance. Importantly, such studies will have to be conducted in conditions as close as possible to farming conditions. However, using metabolites or even enzyme activities as biomarkers is tricky, largely due to their large variations in content in response to even small environmental changes (Florian *et al.*, 2014), including photoperiods (Sulpice *et al.*, 2014). Critically, a large number of metabolites also show large diurnal variations (Flis *et al.*, 2019). Such variability in response to environmental changes and harvest time can lead to difficulties when working under field conditions (Box 1). Thus, if the identification of biomarkers under a given sub-optimal field condition is important (Melandri *et al.*, 2019), multiple field trials under sub-optimal field conditions will be necessary for the identification of robust markers in order to breed for stress tolerant varieties. It will require large collaborative efforts between the fundamental and breeding communities and critically the development of common phenotyping protocols and databases in order to develop comprehensive predictive breeding models (Alexandersson *et al.*, 2014; Sulpice and McKeown, 2015).

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