Commentary

Epigenomics in trees: decoding the plastic lives of mangroves

A draft assembly of the *Bruguiera gymnorhiza* genome facilitates first epigenomic insights into the plastic lives of mangroves

Trees are among the longest-living organisms on Earth. During their long lives, they need to weather numerous environmental challenges, including extreme heat, frost, pests, drought and changes in soil composition. Exposure to these stressors can last anywhere from a few hours to several decades. In most cases, trees manage to overcome these challenges by mounting remarkably flexible stress responses (Gratani, 2014). These often involve substantial morphological and physiological transformations, a phenomenon known as phenotypic plasticity. Decoding the molecular basis underlying this phenomenon is tremendously challenging. In this issue of *New Phytologist*, Miryeganeh *et al.* (2022, pp. 2094–2110) take a genomics approach to identify epigenetic signatures of salt-stress induced phenotypic plasticity in the mangrove species *Bruguiera gymnorhiza* (Fig. 1), one of the most widely distributed mangroves in the Asian Pacific region.

'This species' relatively small, moderately complex, diploid genome, along with its self-compatible nature and extraordinary plasticity could make it a valuable resource for decoding the molecular basis of stress tolerance and phenotypic plasticity in trees.'

The authors examined a population of *B. gymnorhiza* in Okinawa, Japan (Fig. 1a). Trees in this population display a drastic phenotypic dimorphism depending on their local growth conditions (Fig. 1b). Individuals living in high salinity soils along the coastal region (saline) show a dwarfed, shrub-like phenotype and small leaves, while trees growing in brackish soils along a river bank (brackish) only *c.* 1 km away are much taller, have thicker trunks and larger leaves (Fig. 1b). These morphological differences are so pronounced that it is difficult to tell, at first sight, whether saline and brackish individuals actually belong to the same species.

To begin to understand these phenotypic differences at the molecular level, Miryeganeh *et al.* aimed to compare genome-wide gene expression and epigenetic profiles of saline and brackish individuals. However, this required that they first generate an assembly of *B. gymnorphiza* genome, which was only available for two distantly related mangrove species at that time. They accomplished this by using a combination of short- and long-read sequencing technologies. Although the assembly is still in a draft stage (1079 scaffolds, N50 = 2.3 Mb), it did reveal a relatively small, diploid genome (*c.* 309 Mb), containing *c.* 35 000 protein coding genes, and a moderate proportion of transposable elements (TEs) and unclassified repeats (48% of the genome). These statistics compare well against those of other tree genomes, which are often much larger and more complex (Sow *et al.*, 2018).

Using their new assembly as a starting point, Miryeganeh *et al.* measured gene expression profiles of saline and brackish individuals. They uncovered thousands of differentially expressed genes, including genes known to be involved in saline response. These expression differences could be verified in an elegant laboratory experiment where they grew seedlings (derived from seeds of coastal individuals) under two salinity concentrations that mimicked those seen in the natural environments. This result demonstrated that it is the salinity differences, rather than some other factor, that distinguishes individuals from coastal and river environments, at least at the transcriptional level.

It is well known from other plant species that stress-induced gene expression changes often co-occur with, or are mediated by, changes in epigenetic modifications (Lämke & Bäurle, 2017). These epigenetic changes help to establish new gene expression patterns and to maintain them through mitotic cell divisions. They are believed to be key in regulating phenotypic plasticity, particularly in long-lived organisms with complex life cycles such as trees (Bräutigam *et al.*, 2013). One epigenetic modification that seems to be required for this is DNA cytosine methylation. In plants, cytosine methylation occurs in three sequence contexts: CG, CHG and CHH, where H is any nucleotide but G. Heavy methylation in all three contexts is typically found in TEs and repeat sequences. Genes, however, are commonly seen in three types of methylation states: unmethylated, CG-only methylated, or TE-like methylated (Kawakatsu, *et al.*, 2016). Cytosine methylation typically acts as a transcriptional repressor, except for in CG-only methylated genes where its function remains elusive.

Miryeganeh *et al.* compared DNA methylation between saline and brackish individuals. They uncovered tens of thousands of differentially methylated regions (DMRs) throughout the genome. Most notable was the strong hypermethylation of CHH and CHG sites in TEs among saline individuals, which accounted for most of the DMRs, a pattern that could be broadly confirmed in the

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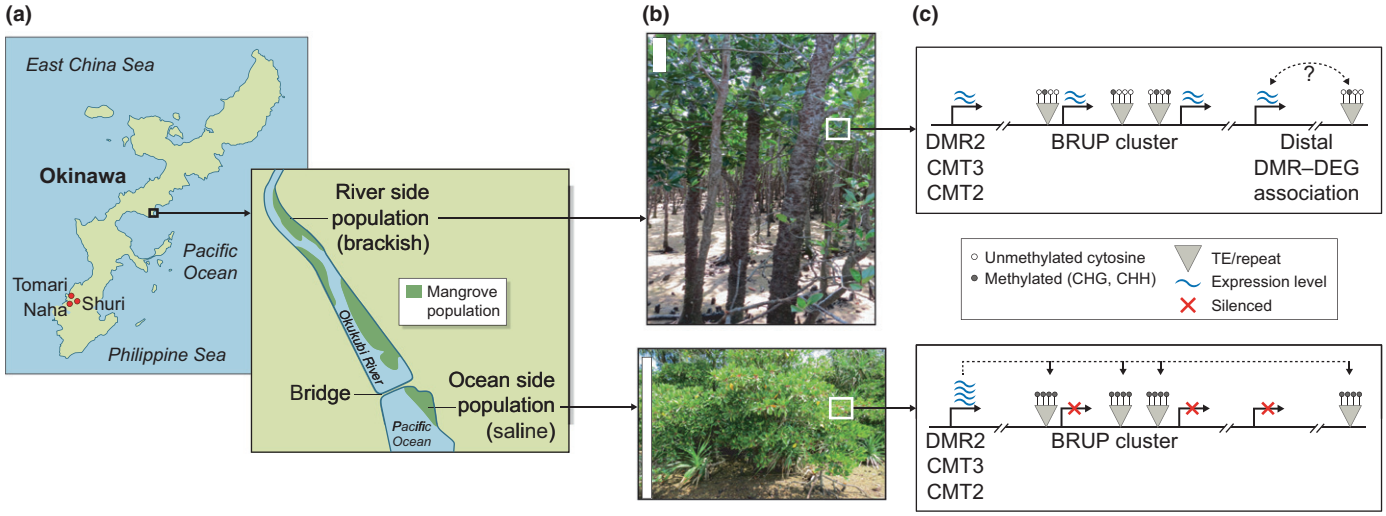

Fig. 1 From Japan's geography to the mangrove cell nucleus. (a) Sampling of mangroves (*Bruguiera gymnorhiza*) along the oceanside (saline soil) and the riverside (brackish soil) on the island of Okinawa, Japan. The two sampling locations are *c*. 1 km apart. (b) *Bruguiera gymnorhiza* shows remarkable phenotypic plasticity: there are strong morphological differences between trees from the saline and brackish environments. The white vertical bar denotes the height of the ocean side individuals. Images from the article in this issue of *New Phytologist* by Miryeganeh *et al*. (2022, pp. 2094–2110). (c) Comparison of gene expression and DNA methylation using the newly assembled *B. gymnorhiza* genome. Trees in the saline environment display genome-wide hypermethylation of mainly transposable elements (TEs) and repeats. Most notable is the hypermethylation of a BRUP gene cluster, which leads to cluster-wide gene silencing. The BRUP cluster has been previously implicated in plant metabolic pathways and saline stress response, and is evolving rapidly in mangroves. The genome-wide hypermethylation seems to be driven by an upregulation of known DNA methylation genes. Most of the detected differentially methylated regions (DMRs) could not be linked to differentially expressed genes (DEGs) in *cis*, raising questions about their regulatory impact. One hypothesis is that many DMRs associate with DEGs in *trans*, possibly via distal *cis* regulatory elements, or through *trans*-acting small RNAs. This hypothesis could be explored in future studies using chromatin interaction maps, along with small RNA profiling. This will require a deeply annotated chromosome-level assembly.

laboratory controls. By contrast, only a small fraction of DMRs were located in and around genes. Attempts to link these genic DMRs to gene expression changes failed. The exception was a *c*. 300 kb cluster of 13 genes encoding BRUP-domain containing proteins. Previous work had already shown that such proteins are involved in various plant metabolic pathways and are downregulated in response to high-salinity conditions (Miyama & Hanagata, 2007). Interestingly, this gene cluster also harbored numerous TEs and other repeats. These TEs were strongly hypermethylated in CHH under saline conditions in both natural and control environments, and were associated with cluster-wide gene silencing (Fig. 1c). It is possible that these TEs have been co-opted to function as environmentally-sensitive *cis*-regulatory elements (Chuong *et al*., 2017).

The BRUP cluster presents a promising target for future functional studies of salt-induced phenotypic plasticity in *B. gymnorhiza*. The fact that this cluster is evolving rapidly, with major expansions, contractions and rearrangements among related mangrove species, seems to indicate that it has a role in adaptive evolution. With the new genome assembly in hand, future work could easily assess if differential methylation patterns within this cluster and/or structural variations (presence/absence variations of repeats, TEs and genes) are associated with differential plasticity (i.e. reaction norms) within and between populations of this species.

Beyond this, there is still tremendous potential to further integrate the detected CHH and CHG DMRs in TEs/repeats with gene expression changes at the genome-wide scale. This is a major challenge, not just in *B. gymnorhiza*, but in most plant species. The problem is that DMR-gene expression associations do not exclusively occur in *cis*, but also involve distal regulatory interactions (Lu *et al*., 2018) (Fig. 1c). One mechanism is through chromatin looping. Differential methylation of distal regulatory elements (enhancers), for example, can alter gene expression states at their loop targets, as shown in maize (Xu *et al*., 2020). Other sources of distal interactions may involve *trans*-acting small RNAs that originate from differentially methylated heterochromatic regions and induce differential expression at their target genes (Chuong *et al*., 2017). Identifying such distal regulatory elements and their targets will require chromatin interaction maps along with a deeply annotated genome. For this to work, a high-quality, chromosome level assembly will be ultimately required.

Such interaction maps can provide a framework for dissecting the (causal) relationship between DNA methylation and gene expression dynamics in response to salt-stress. It is unclear if differential methylation is established first, followed by differential expression, or if methylation changes arise in an expression-dependent manner (Secco *et al*., 2015). Interestingly, the authors show that the hypermethylation in CHH and CHG contexts correlates with the upregulation of homologs of CMT2, DMR2 and CMT3, which are genes known to be involved in CHH and CHG methylation (Law & Jacobsen, 2010), as well as with the downregulation of a homolog of the DNA demethylase DME2, which is responsible for active methylation removal (Penterman *et al*., 2007) (Fig. 1c). Together, this would suggest that differential expression of DNA (de)methylation genes drive the observed hypermethylation. The mechanism by which these DNA (de) methylation genes themselves are differentially regulated in

response to salt stress remains elusive. However, accumulating work shows that chromatin modifiers can interact directly with hormonal pathways (Ojolo *et al.*, 2018), and could therefore act as upstream players in the stress signaling cascade.

Bruguiera gymnorhiza may emerge as a powerful (experimental) model to test such hypotheses. This species' relatively small, moderately complex, diploid genome, along with its self-compatible nature and extraordinary plasticity could make it a valuable resource for decoding the molecular basis of stress tolerance and phenotypic plasticity in trees. The work by Miryeganeh *et al.* provides a first step in this direction.

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