Transgenics in wheat

Transformation of wheat can be performed using a variety of methods including both particle bombardment (Vasil *et al.*, 1992; Sparks and Jones, 2009) and Agrobacterium-mediated transformation (Cheng *et al.*, 1997; Sparks *et al.*, 2014). The generation of stable transformants in wheat most commonly involves the transformation of immature wheat embryos and subsequent callus regeneration (Harwood, 2012). Gene expression can thus be altered in a variety of ways using transgenic approaches such as overexpressing or ectopically expressing the gene of interest using either constitutive, tissue-specific or inducible promoters (Hensel *et al.*, 2011). Similarly, RNA-interference (RNAi) has also been used successfully in wheat to reduce/eliminate gene expression with the added benefit that constructs can be designed to target all homoeologous genes simultaneously, thereby overcoming the issue of functional redundancy (Fu *et al.*, 2007). In addition to altering expression patterns, modified proteins can also be introduced (e.g. including tags) that can be used for downstream experiments, such as ChIP-seq (Deng *et al.*, 2015) or localisation studies (Harwood *et al.*, 2005), although these are still not commonly employed in wheat. As transformation methods have only been optimised for a limited number of wheat varieties it is important to understand whether the gene is expressed/functional in the chosen variety when defining transgenic strategies. Transient gene silencing through Virus Induced Gene Silencing (VIGS) has also be performed in wheat primarily to investigate disease resistance. VIGS has been carried out in a range of varieties, but being restricted to a few tissue types it has been used mostly in leaf tissues (Lee *et al.*, 2015), young seedlings (Zhang *et al.*, 2017a) and spikes (Ma *et al.*, 2012).

In addition to these more traditional transgenic approaches, the recent developments in genome editing technologies provide new opportunities for manipulating genes in wheat. Cas9-mediated genome editing has been successfully demonstrated in wheat both in transient expression systems (Shan *et al.*, 2014) and stably transformed plants (Wang *et al.*, 2014), using a range of methods (reviewed in (Uauy *et al.*, 2017)). Currently, most studies have introduced specific point mutations or small deletions leading to subsequent protein disruption, although the technology holds the potential to have more complex applications such as allele swapping or gene insertion in the future (Puchta, 2017). Similar to RNAi, constructs for Cas9-mediated gene editing can be designed to target all homoeologs simultaneously (Zhang *et al.*, 2016). Due to the current efficiency of genome editing however, the likelihood of obtaining mutations in all homoeologs in a single T_0 plant remains low and subsequent crosses to stack edits are likely required. One of the major limitations of using transgenic approaches to manipulate agronomically relevant traits are the associated regulatory constraints. To overcome this, the nuclease transgene can be segregated away from the edited gene(s) in subsequent generations and studies have also documented methods of Cas9-editing in wheat that avoid transgene integration altogether (Liang *et al.*, 2017).