

Application of next generation sequencing technologies with reference to plant science

Gayatri Gouda¹ and Manoj Kumar Gupta²

Received October 1, 2015 and Accepted January 23, 2016

All disciplines which are DNA sequence data dependent have shown rapid advancement in the last few years because of the development and emergence of next generation sequencing (NGS) technologies. Earlier to NGS technology for almost two decades, the automated Sanger method had conquered the industry and led to a number of enormous achievement, including the completion of the human genome sequence (Metzker *et al.*, 2010; Unamba *et al.*, 2015; Roy *et al.*, 2016). The automated Sanger sequencing method is considered as a first-generation technology while present methods are referred to as next-generation sequencing (NGS). These newer technologies is constituted of various strategies that rely on a combination of template preparation, sequencing, imaging, genome alignment and assembly methods. The arrival of NGS technologies brought change on scientific approaches in basic, applied and clinical research (Metzker *et al.*, 2010; Egan *et al.*, 2012; Kelleher *et al.*, 2015; Unamba *et al.*, 2015; Zhang *et al.*, 2015; Fu and Guole, 2016; Okuno *et al.*, 2016; Vivien *et al.*, 2016). The potential of NGS is similar to the first phase of PCR in some respects with one's thoughts being the primary limitation to its use. The major advantages offered by NGS is the ability to generate a massive volume of data cheaply and in some cases more than one billion short reads per instrument run. This feature expands the monarchy of experimentation beyond just shaping the order of bases. For example, microarrays studies for gene-expression are now being replaced by seq-based methods, which is capable to identify and quantify rare transcript capable to identify and quantify rare transcripts without prior knowledge

of a particular gene and can also provide information regarding alternative splicing and sequence variation in identified genes. The ability to sequence the whole genome of many related organisms by NGS technologies has led to the large-scale comparative and evolutionary studies to be performed that were unimaginable just a few years ago (Metzker *et al.*, 2010; He *et al.*, 2012; Suter *et al.*, 2015; Angelini *et al.*, 2016; Lancini *et al.*, 2016; Okuno *et al.*, 2016; Roy *et al.*, 2016; Vrancken *et al.*, 2016). The broadest use of NGS may be the re-sequencing of human genomes to boost our understanding of how genetic differences affect health and disease. In agriculture, NGS technology is used with model organisms of economic importance during the initial stage. Azam *et al.* (2012) have done comparative study using four short read alignment tools (Maq, BowTie, Novoalim and SOAP2) with their new approach called coverage-based consensus called (CbCC) for SNP discovery in chickpea, *Cicer arietinum* L., a crop lacking a reference genome. Maq was found to be most accurate and sensitive, even at low read depth. Greater accuracy was demonstrated by all four tools at higher read depth, and SNPs predicted by three or four tools were more likely to be correct. SNP prediction accuracy normally increased with the increase in read depth. The results obtained in this study are applicable for NGS-based SNP discovery in any other plant species that does not have a reference genome. In addition, 4543 putative SNPs have been identified in chickpea that will be useful for advancing chickpea genetics research and breeding applications. (Azam *et al.*, 2012). 22 EST libraries was generated by Lai *et al.* (2012) from a variety of tissue for 11 weeds in the

sunflower family, using Illumina sequencing and Sanger 454. They also compared the coverage and quality of sequence assemblies, and developed Nimble Gen microarrays for expression analyses in five of them (Lai *et al.*, 2012).

In this review we will discuss about different existing NGS technologies and their use in plant biology.