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Next generation sequencing technology for development of genomic simple sequence repeat (SSR) markers for linseed

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Linseed (*Linum usitatissimum* L.), is regarded as a cash crop of tomorrow because of the presence of nutraceutically important α-linolenic acid (ALA) and lignan. However, due to the lack of sufficient genetic and genomic resources, only limited breeding progress has been made in this crop. For diversity analysis, genetic mapping and tagging traits, simple sequence repeat (SSR) markers are favored because of their co-dominant and highly polymorphic nature. To develop SSR markers for linseed, microsatellites enriched genomic libraries were constructed using three methods *viz.* PCR Isolation of Microsatellite Arrays (PIMA), 5' anchored PCR method and Fast Isolation by AFLP of Sequences COntaining repeat (FIASCO). Amplified products from the three methods were pooled and sequenced using 454 GS-FLX instrument and Titanium chemistry. Eighteen hundred and forty two microsatellite motifs were identified from 2,183 contigs and 2,509 singlets, assembled from 36,332 reads obtained. Dinucleotide repeats were most abundant (54%) followed by trinucleotide (44%) repeats. Two hundred and ninety primer pairs were developed, 52 of which were evaluated using a panel of 27 diverse linseed genotypes. The 5'anchored method was most efficient for isolation of microsatellites among the three enrichment methods, although the FIASCO method was more efficient for development of SSR markers. These markers may be used for diversity analysis, development of linkage map and tagging the genes and traits in linseed.