In plants, microRNAs (miRNAs) play significant roles in post-transcriptional gene regulation and have been found to control many genes involved in different biological and metabolic processes. Extensive studies were carried out to discover miRNAs and analyze their functions in model plant species, such as in Arabidopsis and rice that have been reported. In this research, we used bioinformatics to predict microRNAs in an important strawberry rootstock cultivar to discover and validate precise sequences of microRNAs in strawberry. By adopting a range of filtering criteria, we obtained 59 potential miRNAs belonging to 40 miRNA families from the Fragaria vesca genome. Using two specific 5' and 3' miRNA RACE PCR reactions and a sequence-directed cloning method, we accurately determined 34 precise sequences of candidate miRNAs, while six other sequences exhibited some minor divergence in their termini nucleotides, and 19 miRNAs that could not be cloned owing to expression abundance may be too low or these mirRNAs predicted could not be existing in strawberry. Potential target genes were further predicted for the miRNAs above. The expression of the 16 miRNAs unreported and having exact sequences and their targets by experiment could be detected in different tissues of strawberry ranging from roots, stems, leaves, flowers and fruits by qRT-PCR and some of them showed differential expression in various tissues. The functional analysis of 16 miRNAs and their targets was carried out. Finally, we conclude that there are 34 mirRNAs in strawberry and their targets play vital roles not only in growth and development, but also in diverse physiological processes. These results show that regulatory miRNAs exist in agronomically important strawberry and might have an important function in strawberry growth and development.