During their lifetime, plants require nitrogen (N) to grow and develop. While most species depend on bioavailable soil N, legumes are able to host N₂-fixing rhizobia in specialized root-derived structures called nodules. Rhizobia initially colonize legume roots via infection threads in root hairs. The model legume *Lotus japonicus* develops nodules from a limited number of cortex cells and only few root hairs are capable of being infected by rhizobia through infection threads. The limited number of these cells impedes the detection of specific transcriptional signatures in classical bulk RNA-seq approaches. However, they might be crucial to get novel insights into nodule organogenesis and infection thread formation. By performing protoplast-based single-cell RNA-seq (DROP-seq), we identified not only cell populations of distinct root tissues but also those representing infected root hairs and cortex cells as well as nodule cells.