From the perspective of nitrogen metabolism, the accumulation of proline, soluble proteins, and related amino acids in millet under stress is not simply an increase in "protective substances." It also reflects the shift of nitrogen allocation from structural growth to osmotic adjustment and stress defense. Ravichandran et al. (2025), through comparative transcriptomic and metabolic pathway analyses of foxtail millet and broomcorn millet under salt stress, showed that both millet crops activate complex metabolic interactions, enzyme activity changes, and transcription factor regulation under stress conditions. Amino acid metabolism, antioxidant defense, and energy metabolism jointly participate in stress responses. Therefore, nitrogen metabolic reprogramming in broomcorn millet should be regarded as a "growth-defense trade-off": part of the nitrogen is used to synthesize proline, soluble proteins, and stress-related enzymes, rather than being fully deposited into grain storage proteins.

As a result, grain protein changes in saline-alkali environments may occur in two directions. First, yield reduction may lead to a relative concentration of protein in the grains. Second, severe stress may suppress grain filling and nitrogen transport, thereby limiting protein deposition. In agricultural applications, evaluation under saline-alkali conditions should not focus only on yield. Grain crude protein, free amino acids, proline, glutamine synthetase activity, and nitrogen harvest index should also be measured simultaneously. Only by combining "yield stability" with "nitrogen metabolic quality" can truly suitable broomcorn millet materials for saline-alkali land utilization be identified.

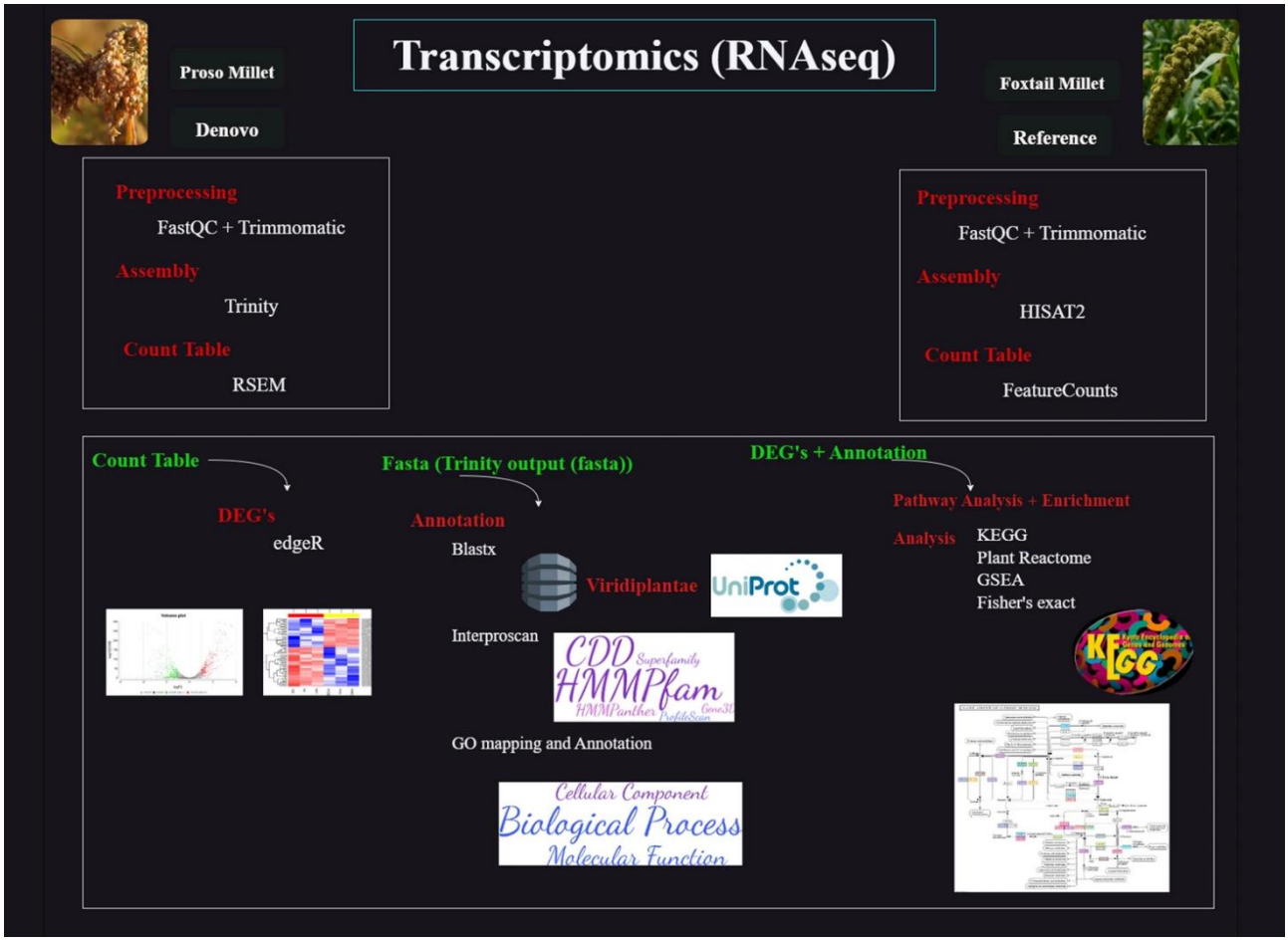

Figure 2 A detailed workflow of data collection, processing, analysis, and visualization used in this study for identifying and evaluating important transcription factors and pathways in foxtail and proso millets under salt stress (Adopted from Ravichandran et al., 2025)

5.2 Accumulation of bioactive compounds

Alkaline stress not only changes yield-related traits, but also affects the metabolite composition in broomcorn millet grains. Using two millet varieties, S223 and T289, Ma et al. (2023b) analyzed the effects of alkaline stress on non-volatile and volatile metabolites in mature grains. A total of 933 non-volatile metabolites and 313 volatile