The caffeine biosynthetic pathway is composed of three methylation steps, and \( \text{N} \)-methyltransferase catalyzing each step has high substrate specificity. Since the amino acid sequences among coffee 7-methylxanthosine synthase (\( \text{CmXRS1} \)), theobromine synthase, and caffeine synthase are highly homologous to each other, these substrate specificities seem to be determined in a very restricted region. The analysis of site-directed mutants for \( \text{CmXRS1} \) that naturally acts at the initial step, \textit{i.e.} 7-\( \text{N} \) methylation of xanthosine, revealed that the activity of 3-\( \text{N} \) methylation needs a histidine residue at corresponding position 161 in the \( \text{CmXRS1} \) sequence. We succeeded in producing the mutant enzyme which can catalyze the first and second methylation steps in caffeine biosynthesis.

**Key words:** Coffee, Caffeine, \( \text{N} \)-Methyltransferase