Chapter-I

Introduction
The amino acid biosynthetic pathway has gained currency as metabolic engineering of the pathway could lead to production of higher level of essential amino acids in transgenic plants. The essential amino acids lysine, threonine, isoleucine and methionine are not synthesized in mammals. Therefore, biosynthesis of these amino acids in plants is of great interest because of their importance in human and animal diet. These essential amino acids are synthesized from aspartate via the aspartate family biosynthetic pathway (Fig. 1).

![Aspartate family amino acid biosynthetic pathway](image-url)

Fig. 1: Aspartate family amino acid biosynthetic pathway
Aspartate kinase, also known as aspartokinase (AK, EC 2.7.2.4) controls the flux of 4-carbon through this pathway by converting aspartate into β-aspartyl phosphate, which in turn, is converted into aspartate semialdehyde. The pathway is divided at this point: one branch leads to the formation of lysine and other to the formation of threonine, isoleucine and methionine (Bryan, 1980; Galili, 1995). One reaction in the branch is the reduction of aspartyl semialdehyde to homoserine by the enzyme homoserine dehydrogenase (HSDH, EC 1.1.1.3) and another reaction is the formation of 2,3 dihydrodipicolinate by the enzyme dihydropicolinate synthase (DHPS, EC 4.2.1.52).

This pathway in higher plants is regulated by its end products. The aspartate kinase is feed back inhibited by both lysine and threonine. In addition, lysine inhibits the activity of dihydropicolinate synthase, whereas threonine inhibits the activity of homoserine dehydrogenase. Therefore, in addition to feedback inhibition, lysine and threonine also inhibit the activity of enzymes involved in their own biosynthesis at the branch point.

Plants are reported to possess several distinct AK isozymes that are sensitive to feedback inhibition by either lysine or threonine or both (Galili, 1995). In recent years there has been a great interest in amino acid biosynthetic genes as that could be employed to enhance the amino acid content in the storage organs or vegetative parts by expressing the desensitized form of these biosynthetic enzymes. High level expression of the desensitized form of a bacterial AK gene in transgenic tobacco plant was shown to increase the amino acid pool (Shaul and Galili, 1992; Karchi et al, 1993). Because of the nutritional importance of lysine and threonine it is essential to study extensively the biochemical and molecular characteristics of these biosynthetic genes in different plants. In the
present investigation, an attempt has been made to study the aspartate kinase in *Cicer arietinum* L. (chickpea), an important legume in India. Cloning of the isozyme specific gene of AK and its ectopic expression in transgenic plants will help in elucidating the cellular and developmental regulation of AK. Therefore, an attempt has been made to study the isozyme pattern of AK and to purify them. The cloning of aspartate kinase from *C. arietinum* L. has also been tried.

Phytochrome, a photoreceptor, functions as a binary molecular switch controlling the plant gene expression in response to light. The photoreceptor is reversibly interconvertible between its inactive form (Pr) and active form (Pfr). The Pfr formation initiates the signalling cascade, which culminates in altering gene expression. An attempt has also been made to understand how aspartate kinase is regulated by phytochrome. The phytochrome signalling pathway is mediated by Ca\(^{2+}\) /calmodulin (Neuhaus et al, 1993), cyclic GMP (Bowler et al, 1994) and 1,4,5-inositol triphosphate (IP\(_3\), Raghuram and Sopory, 1995). Therefore, an attempt has also been made to look into how phytochrome-induced signalling cascade is orchestrated to activate/induce aspartate kinase.

Various intra-cellular molecules such as Ca\(^{2+}\), cyclic AMP, IP\(_3\), protein kinases and phosphatases are known to be involved in a complex network to orchestrate the signalling cascade in plants (Lessard et al, 1997; Mulligan et al, 1997). The activation or inhibition of various metabolic and catabolic pathways are also mediated through this cascade (Saito et al, 1994; Nimmo et al, 1990; Bakrim et al, 1992). Therefore, experiments were performed to understand how the aspartate family pathway is influenced by different signalling molecules such as Ca\(^{2+}\), protein kinases and phosphatases.