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Asparagus, the type genus of the monocot family Asparagaceae includes approximately 300 species distributed from sub-arid to arid regions in the Old World (Dahlgren et al. 1985; Clifford and Conran 1987). It is characterized by varied forms of modified photosynthetic stems (cladodes). Different species of the genus Asparagus are reported to have high medicinal (Kar and Sen 1985; Kanno and Yokoyama 2011) and ornamental values (Kanno and Yokoyama 2011). The genus includes diverse life forms, such as herbaceous perennials, tender shrubs and woody vines. Dahlgren and Clifford (1982) delimited the family Asparagaceae in narrow sense and accepted three separate genera of Asparagus s.l. viz. Asparagus s.s., Asparagopsis and Myrsiphyllum, and also included other three genera: Ruscus, Danae and Semele, within Asparagaceae. Obermeyer (1983) followed Dahlgren and Clifford’s classification (1982) and proposed the generic name Protasparagus Oberm. to replace the homonym Asparagopsis Kunth with 38 new combinations of species (Obermeyer 1983). Later on, Dahlgren et al. (1985) transferred Ruscus, Danae and Semele into the separate family Ruscaceae, on the basis of leaf, floral morphology and phytochemistry and therefore, reported Asparagaceae with only three genera: Asparagus, Myrsiphyllum and Protasparagus. But they also admitted that the later two might be best treated as subgenera (Dahlgren et al. 1985).
The most recent infrageneric classification system divides the genus *Asparagus* into three subgenera: *Asparagus*, *Myrsiphyllum*, and *Protasparagus* (Clifford and Conran 1987). The subgenus *Asparagus* comprises all dioecious species, whereas the *Myrsiphyllum* consists of hermaphroditic, shortly united tepals with connivent filaments and the remaining hermaphroditic species with free filaments are grouped within the subgenus *Protasparagus* (Malcomber and Demissew 1993). However, many members of *Protasparagus* and *Asparagus* are found to share similar morphological characteristics resulting in complicated taxonomic relationships (Malcomber and Demissew 1993). Obermeyer (1983) suggested that species of *Protasparagus* are primitive on the basis of morphological characteristics such as bisexual flowers, many ovules per locule and globular seeds. Obermeyer and Immelman (1992) characterized this subgenus into eight diverse series on the basis of morphological and floral characters, while Malcomber and Demissew (1993) suggested that the floral characters were inconsistent to demarcate this subgenus. Hence, the interspecific delimitation of the subgenus *Protasparagus* is ill-defined till date due to lack of discontinuous morphological characters. Members of *Protasparagus* are mainly concentrated in Southern to tropical Africa, Asia and extended to Australia. Nevertheless it is also reported that such widespread *Protasparagus* species were not often collected, probably because of the short flowering period and troublesome spines (Obermeyer and Immelman 1992).

A few phylogenetic studies (Lee et al. 1997; Stajner et al. 2002; Fukuda et al. 2005; 2012; Kubota et al. 2012) have been conducted so far, inferring partly the phylogenetic status of the subgenus *Asparagus* and *Myrsiphyllum* while the interspecific relationship within *Protasparagus* remained unresolved. Among these few studies on *Asparagus*, Lee
et al. (1997) suggested that the subgenus *Asparagus* is monophyletic in origin based on RFLP analysis of cpDNA. Stajner et al. (2002) conducted a phylogenetic study based on RFLP analysis of the rDNA ITS region in ten *Asparagus* species and revealed that European and African species comprise distinct monophyletic groups. Very recently, Fukuda et al. (2005) and Kubota et al. (2012) distinguished *Myrsiphyllum* from the subgenus *Asparagus* based on cpDNA sequence and rDNA ITS1 sequence but failed to resolve the phylogenetic status of *Protasparagus*. Therefore, in order to address the complete phylogenetic status of the genus *Asparagus*, it is important to explore the interspecific relationships particularly within the members of the *Asparagus* subgenus *Protasparagus* through comprehensive molecular systematic studies.

The use of nuclear ribosomal DNA (rDNA) and chloroplast DNA (cpDNA) sequence in plant molecular systematic studies has been reported to elucidate the relationships among closely related taxa (Fukuda et al. 2005; Nishizawa and Watano 2000; Alvarez and Wendel 2003; Peterson et al. 2004; Castro et al. 2013). Thus, the present study analyzed both the nuclear rDNA ITS regions (ITS1-5.8S-ITS2) and cpDNA *trnL* intron sequence complexity.

Though molecular systematic studies based on nucleotide sequences provide the most useful data in analyzing the phylogenetic relationships between the lower taxonomic ranks, chromosome number and nuclear DNA content can also be used as taxonomic markers in species relationships. The genus *Asparagus* is reported to have a basic chromosome number (x= 10) and includes species of different ploidy levels (Kar and Sen 1985; Sheidai and Inamdar 1997). The polyploidy is a key factor in higher plant evolution leading to the formation of a new species (Abbott and Lowe 2004; Soltis et al. 2004).
Only a few cytological studies with ploidy estimation have been reported in the genus *Asparagus* and majority of which is completely based on chromosome number counts. (Kar and Sen 1985; Sheidai and Inamdar 1997; Vijayavalli and Mathew 1990). Recently, the introduction of flow cytometry into plant genetic studies improves ploidy level determination and establishment of DNA amounts. It has been successfully applied for the estimation of nuclear DNA content in some species of *Asparagus* (Stajner et al. 2002; Moreno et al. 2008; Zenkteler et al. 2012). The nuclear DNA C-value in plants is an important genotypic trait for a taxon and is very effective in delimiting the interspecific relationships (Ohri 1998). Therefore, to explore the interspecific relationship between the members of the subgenus *Protasparagus*, the present study analyzed both the chromosome numbers and the nuclear DNA content.

Since *Asparagus* and only a few genera of angiosperms (*Ruscus, Danae*, and *Epiphyllum*) are characterized by cladodes (Cooney-Sovetts and Sattler 1987), different cladode traits especially cladode shape, cladode numbers per axil and several cladode anatomical characters have been used as important taxonomic markers (Baker 1875; Arber 1924; Guvenc and Koyuncu 2002). Similarly, the importance of stomatal characters is well established in several comparative anatomical and phylogenetic studies as evolutionary markers at various levels of systematic hierarchy (Metcalf 1961; Van-Cotthem 1970; Tripathi and Mondal 2012). Therefore, the present study also investigated different cladode morphological as well as anatomical and stomatal characters which provide additional phylogenetic information about this subgenus.

On the other hand, several studies have provided the evidence of the usefulness of different phytochemicals to support the molecular based phylogenies at various taxonomic
levels (Grayer et al. 1999; Jensen et al. 2002; Ronsted et al. 2003). The genus *Asparagus* is also well known for its immense medicinal uses because of the presence of diverse type of phytochemicals including saponins saponidins (Kar and Sen 1985; Kanno and Yokoyama, 2011). Steroidal saponins or sapogenins have been used as chemotaxonomic markers for the genera *Asparagus* and also for *Ruscus* (Hegnauer 1986). Therefore, the present study analyzed the two important principal secondary metabolites i.e., shatavarin-IV and sarsasapogenin of different members of the subgenus *Protasparagus* in order to investigate their phylogenetic significance.

**Therefore, the aim of the present study was to explore the molecular phylogenetic relationships within the members of *Asparagus* subgenus *Protasparagus*.**