Chapter 1.1

**LOTUS JAPONICUS AS A MODEL SYSTEM**

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Lotus japonicus was proposed as a model legume twelve years ago, because of a number of characteristics making this system very amenable for legume research. These characteristics include small plant, large and abundant flowers, easy hand pollination, high seed production, short generation time, easy cultivation, amenable to plant transformation and regeneration from tissue culture. At present, a set of genetic resources and tools has rapidly become available, including ecotypes, mutant lines, genetic maps, RIL lines, transformation procedures, EST sequences, and a whole genome-sequencing project. In these twelve years, research on L. japonicus has greatly contributed to the understanding of both symbiotic processes, i.e. with Rhizobium and mycorrhiza, making possible the cloning of several key genes involved in both symbioses. Now Lotus is regarded as one of the most useful plants for legume study and researchers who have interests in nodulation and other aspects of legume biology use it worldwide. In this introductory chapter we deal with the most general aspects and possibilities of the biology of L. japonicus, plant growth conditions, culture media, nitrogen supply and symbiotic partners; plant tissue culture; genetic transformation and regeneration of transgenic plants, contribution to the understanding of symbiotic processes and the role of this model plant for other research topics and exploiting microsynteny.

**INTRODUCTION**

The family Leguminosae (Fabaceae), which is the third largest Angiosperm family, contains a number of important crop plants and woody trees, producing protein, carbohydrates, and oil for human consumption and animal fodder. Legumes are ‘pioneer’ plants that are able to grow in nutrient-poor soils by virtue of their ability to establish symbioses with nitrogen-fixing rhizobia bacteria, and with nutrient scavenging soil fungi, which provide the plants with phosphorous and other essential
nutrients. These natural features favoured the adoption of legumes in ancient agriculture, and make them an important part of sustainable agricultural systems to this day.

Among the plant families, symbiotic interaction with rhizobia is unique to legumes. The only known exception is *Parasponia andersonii* belonging to the elm family. Another symbiosis providing nitrogen to host plants is between gram-positive bacteria *Frankia* and several woody plant species forming actinorhizal symbiosis. Phylogenetic analysis based on DNA sequencing of the *rbcL* genes places all plants involved in rhizobial or actinorhizal symbiosis in the same clade (Rosid I), thus indicating that predisposition for nodulation evolved only once (Soltis et al, 1995, Doyle, 1998). However, different morphology and biochemistry of several types of nodules, indicate multiple origins of nodulation, perhaps even within the legume family (Doyle, 1998; Doyle and Luckow, 2003). The symbiotic interaction of legume plants with mycorrhizal fungi, though it is not exclusive to this family, is also an important interaction, which provides phosphorus and other nutrients to the plant. Obtaining sufficient nutrients from the soil is a common problem facing land plants, and 80% to 90% of all land plants are assisted in nutrient mining through association with symbiotic arbuscular mycorrhizal fungi. Both rhizobial and mycorrhizal symbiosis have been investigated previously, but modern molecular tools have only recently become available to study the genetics and genomics of root symbiosis. Increasing our knowledge of the biology and genetics of the legumes may improve this important agricultural resource as well as complement *Arabidopsis*, which does not develop rhizobial symbiosis or fungal symbiosis leading to vesicular arbuscular mycorrhiza.

**BIOLOGY**

With close to 18,000 different species belonging to the subfamilies Mimosoideae, Caesalpinioideae and Papilionoideae the legume family is a large and diverse plant family that includes several species of agricultural interest. The importance of the family from applied, botanical, and basic scientific perspectives merits a focused effort on a model legume. The poor ability of *Arabidopsis* to enter into symbiotic interaction with mycorrhiza and the inability to form nitrogen fixing root nodules in symbiosis with *Rhizobium* or it allies has been a further incentive to develop a model legume. A clear illustration of the need for "family specific" model plants is the relatively low levels of microsynteny between *Arabidopsis* and *L. japonicus* suggesting that the structure of legume genomes might be quite different from *Arabidopsis*. Recent bioinformatic based studies of genome evolution supports this view and indicates that legumes may not have undergone one of the genome duplication events inferred for *Arabidopsis*. Genomic research on models such as *Lotus japonicus*, should allow a fundamental understanding of the nodulation process and identification of genes involved in nodule formation and mycorrhizal symbiosis. Together with soybean, bean, cowpea, and *Sesbania, L. japonicus* belongs to legumes forming determinate nodules. Cortical cell divisions leading to the formation of the determinate nodule primordium initiate in the outer cortex. A meristem starts dividing to form the nodule primordium, and afterwards the meristematic activity ceases. Nodule size then increases via cell expansion.