4 Transgenic Asparagus (*Asparagus officinalis* L.)

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1 Introduction

1.1 The Plant

*Asparagus officinalis* L. is a perennial monocotyledon, a member of the Liliaceae family. It grows in temperate climates and under subtropical conditions. Growth of spears normally takes place in sandy soils. There are two culture types: the white asparagus, in which the spears are harvested from earthed up plants, where spears are cut at the crown level just as they emerge through the soil, and the green asparagus, in which the spears are harvested at or about ground level when they reach a fixed height above the soil. The main producers are the USA, Spain, France, and Taiwan. White asparagus production predominates in Europe (France and Spain) and Taiwan, while green asparagus is essential in North America and Australasia. In European countries, white asparagus is produced mainly for the fresh market, whereas canned white asparagus is produced in Taiwan.

Asparagus is a dioecious species (*2n = 20*) with a sex ratio of 1:1; however, plants are andromonoecious to hermaphroditic. Male flowers bear six stamens and a rudimentary pistil; female flowers have collapsed anthers surrounding an ovule. Genetic experiments have demonstrated that asparagus female flowers are homogametic (XX), and male and hermaphroditic flowers heterogametic (XY) (Rick and Hanna 1943). The dioecy means that the old common varieties are populations exhibiting a high degree of natural heterozygosity with very irregular yields.

Improvement in asparagus breeding was obtained in two steps with the help of in vitro cell culture techniques. The first step was the production of hybrid varieties: double hybrids (Corriols-Thévenin 1979) and clonal hybrids (Doré 1975; Javouhey 1990). Micropropagation was developed (reviewed by Desjardins 1992) to provide the large number of clones required

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for the parental production of hybrids. The second step was the use of haploids to create F1 hybrids (Thévenin 1968). Haploids are obtained by polyembryony (Thévenin 1968) or in vitro anther culture (reviewed by Doré 1990).

1.2 Need for Transformation

Because of its perennial character, asparagus demands heavy financial input at the plantation stage; plants are productive for 10 years or more, and the benefits are obtained only after 5 or 6 years. Each factor limiting the production and the longevity of the crop is highly damaging.

A disease syndrome known as asparagus decline or crown and root rot, caused by *Fusarium oxysporum* Schlecht f. sp. *asparagi* Cohen and *F. moniliforme* Sheldon, decreases yields and necessitates the removal of fields worldwide (Cohen and Heald 1941; Endo and Burkholder 1971). This disease is the major limiting factor in asparagus production (Grove 1976). The French harvest decreased to 20% in 1989–1990 and 2500ha were removed in 1989–1991. These fungi associated with the Asparagus viruses I, II, or tobacco streak virus also significantly reduce the productivity of asparagus in Mexico.

These diseases have initiated important breeding programs to tackle this problem. Unfortunately, no highly resistant cultivars were available in *Asparagus officinalis*. Crosses with the resistant species *A. sprengeri* (Lewis and Shoemaker 1964) have been unsuccessful, probably due to incompatibility barriers (Elmer et al. 1989). Plant chemical treatments have not been successful, and soil fumigations offer no long-term effectiveness (Lacy 1979). Alternative strategies are needed.

One strategy is to produce transgenic plants which express cloned resistance genes for improving resistance to some pathogens. The first report of success with this approach was transgenic tobacco and rape containing a chitinase with a constitutive promoter. These plants have been shown to exhibit higher basal levels of chitinase and concomitant increased resistance to pathogenic soil fungi when compared with control plants (Broglie et al. 1991). One other gene, encoding a ribosome-inactivating protein in transgenic plants, confers partial protection against fungal attacks (Logemann et al. 1992).

Since higher yield and precocity are correlated with staminate plants (Ellison et al. 1960), there have been efforts in asparagus breeding to produce all male varieties. Regeneration of dihaploids after anther culture is the only way to obtain supermale (YY) plants, homozygous for all characters. Such homozygous plants, crossed with female (XX), produced all male F1 plants (Doré 1974). This research is very complicated, and an alternative could be to tag and select the male plants with a marked gene after transformation of existing varieties.