MOLECULAR BREEDING FOR IMPROVED BIOTIC STRESS RESISTANCE

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Abstract

Molecular breeding of wheat in the sense of genetic engineering will be the subject of this paper. It will review actual strategies for engineering fungal resistance in crops. An efficient method for genetic transformation of wheat like the biolistic method using immature embryos as target tissue – together with genes that directly or indirectly inhibit the pathogen’s spread – form the basis for the development of transgenic wheat plants with improved fungal resistance.

Key words: wheat, transformation, fungal resistance, transgenic strategies

Introduction

Bread wheat *Triticum aestivum* along with rice and maize are the three main important cereals. Nutritionally, wheat covers about 20% of the calorie intake, worldwide. The human population increases about 250,000 people per day, whereas the cultivatable area is decreasing. Therefore the reduction in loss of the yields is a major challenge for crop production in agriculture.

Among the plant diseases, fungi are of great importance, because they cause regular yield losses up to 30% and may reduce the quality of the harvest by mycotoxins. Up to now, the spread of fungal diseases has mainly been controlled in the following three ways: (1) different husbandry techniques as i.e. crop rotation, (2) the use of fungicides and (3) the breeding for resistant cultivars of crops. In spite of the use of fungicides – which reduce profitability - damages rest in the order of 10% (Oerke et al., 1994). The permanently evolving pathogenic fungi species fastly overcome bred resistance or the effects of fungicides.

Classical breeding for resistance is a time-consuming process of crosses and back-crosses. In some cases, this approach will not be realizable because of the lack of natural sources of resistance for important crops like wheat.

This problem can be circumvented by genetic engineering. The combination of reliable *in vitro* culture systems and an efficient method for the transfer of DNA are the prerequisites for genetic engineering of crops. The biolistic transformation method (Sanford et al., 1987) has proved to be a successful method for the transfer of DNA into cereals like maize, rice, wheat, barley, oat and triticale (Gordon-Kamm et al., 1990; Christou et al., 1991; Vasil et al., 1992; Jähne et al., 1994; Somers et al., 1992; Zimny et al., 1995).
Materials and Methods

Detailed description of the in vitro culture system and transformation procedure for the development of stably transformed wheat plants are described by Becker et al. (1994) and by Becker and Lörz (1996).

Results and Discussion

Transgenic approaches in cereals often have their precursor in approaches with dicotyledonous species like transgenic tobacco as a model. The transformation of dicotyledonous species is in general much more simple compared to cereal transformation. The following examples stand exemplary for current approaches with the aim to increase fungal resistance in transgenic plants. They are based on mainly three different strategies:

A. the expression of antifungal proteins,
B. the synthesis of low molecular substances with antimicrobial effects and
C. the induction of an artificial local cell death after infection.

A. Among the antifungal proteins are the so called pathogenesis related (PR) genes which code for coordinatively expressed proteins after a preceded infection (Gianinazzi et al., 1970; Van Loon and Van Kammen, 1970). The hydrolytic enzymes, chitinase and β-1,3-glucanase, capable of degrading fungal the cell wall compounds chitin and β-1,3-glucan are the best studied PR-proteins. Further proteins with antifungal effects like a ribosome-inactivating protein from barley (RIPs) specifically inactivating protein synthesis in fungi or even a fungal protein proved an increased resistance when overexpressing them in transgenic tobacco (Broglie et al., 1991, Gornhardt et al., 1994; Jach et al., 1995). A synergistic resistance effect was observed, when different antifungal proteins were expressed in combination (Jach et al., 1995; Jongedijk et al., 1995; Zhu et al., 1994).

The promising results from transgenic dicots were the basis for increasing the spectrum of defence reactions in wheat by three different antifungal proteins (Table I). For the biolistic transformation of wheat, cDNA-sequences of the chitinase II- and the rip I-gene from barley (Leah et al., 1991) and of the agp-gene from the fungus Aspergillus giganteus (Nakaya et al., 1990) were used. The corresponding gene products are Chitinase II, RIP I and Ag-AFP.

The model in figure 1 explains the underlying idea of the approach.
- Chitinases inhibit the fungal growth by hydrolysing the glycosidic bonds in the polymer chitin and thereby weaken the fungal cell wall. Especially in the hyphal tip, the cell wall can no longer counteract the turgor and the hyphae will lyse.
- Ribosome-inactivating proteins, like the RIP I of barley inhibit the protein synthesis of fungi. These enzymes cleave a specific adenine in the 28 S-RNA of fungal ribosomes and thereby prevent the translation in fungi.
- Ag-AFP is an example of the many proteins with antifungal properties, that do not originate from plants. This fungal protein possesses an amphipathic surface and probably induces the formation of pores in the plasmamembranes of fungi. As a consequence an uncontrollable efflux of ions and metabolites takes place.