Wheat Genetic Transformation as Efficient Tools to Fight against Fungal Diseases

Danielle Christelle Tinak Ekom^{1,3}, Mohammed Nabil Benchekroun¹, Sripada Mahabal Udupa², Driss Iraqi³ and Moulay Mustapha Ennaji¹

1. Laboratory of Virology, Microbiology and Quality/Ecotoxicology and Biodiversity, Faculty of Sciences and Techniques, University Hassan II Mohammedia-Casablanca, P.O. Box 146, Quartier Yasmina, Mohammedia 20650, Morocco

2. ICARDA-INRA Cooperative Research Project, International Center for Agricultural Research in the Dry Area (ICARDA), B.P. 6299, Rabat, Morocco

3. Biotechnology Research Unit, Institut National de la Recherche Agronomique (INRA), B.P. 415, Avenue de la Victoire, Rabat, Morocco

Abstract: Wheat ranks first among cereal crops cultivated in the world. In its production, diseases like powdery mildew, fusarium head blight and rusts caused by fungal pathogens represent a major problem. They produce different symptoms that cause severe crop damage by infecting the spikes, leaves, roots, stems and grains. They are causing losses both by reducing the quantity of the harvested crop and the quality of the product. Quality problems of the harvested product can be due to shrivelled seed, which are frequently found as a consequence of the infection by leaf pathogens, such as mildews, rusts and *Septoria*. Fusarium head blight is the major culprit for mycotoxin contamination from the harvested grain, causing economic losses and in the worst case human and animal health problems. In severe epidemics, all these fungal diseases can significantly reduce yield. Resistance to fungi is beneficial not only from a commercial point of view (yield), but also because of the reduced levels of mycotoxins. The integration of transgenic approaches offers a potential chemical-free and environment-friendly solution for controlling fungal pathogens. This is an essential asset for wheat world food security.

Key words: Crop damages, food security, transgenic approaches, wheat fungal diseases.

1. Introduction

Increased production of major cereal crops like wheat is important for world food security. Wheat is of greater importance in terms of tonnage and financial value [1]. One significant constraint to increased wheat production is a variety of fungal diseases, especially powdery mildew, fusarium head blight and rusts which attack the crop at different stages of its development. These attacks can cause significant losses, when susceptible varieties are used and the environmental conditions are favorable for disease development [2]. Wheat is susceptible to fungal infections, which lower productivity and

deteriorate yield quality [3]. Fungal pathogens of wheat diseases cause symptoms that are specific to them. Therefore, it is important to recognize these symptoms in order to identify each disease. Each disease has specific development conditions. Knowledge of development factors is important in the reasoning of the fight.

Chemical fight, i.e., the use of fungicides, is the most practice. However, genetically modified (GM) wheat engineered for pathogen resistance might be a cost-efficient and ecological alternative to the large use of fungicides [4]. In fact, conventional breeding for resistance to plant diseases through sexual hybridization to transform and polymerize resistant genes shows lower effect because of longer cycle, lack of resistant resources and introduction of

Corresponding author: Moulay Mustapha Ennaji, professor, research fields: virology, microbiology and quality. E-mail: m.ennaji@yahoo.fr.

adverse genes. With the rapid development of plant genetic engineering technologies, molecular breeding with core of cloning and transforming disease resistant and the other related genes has attracted extensive attention [5]. Though there is a great demand for wheat varieties with resistance to diseases, genes conferred with these traits are limited, as they are mostly quantitative traits. Nonetheless, the direct introduction of a small number of genes by genetic engineering offers a convenient and rapid approach for the improvement of stress tolerance [6]. Here, the authors present major fungal diseases of wheat and transgenic wheat plants generated for this purpose.

2. Wheat Fungal Diseases

2.1 Wheat Powdery Mildew

2.1.1 Symptoms of Wheat Powdery Mildew

The symptoms of powdery mildew caused by *Blumeria graminis* f. sp. *tritici* (Fig. 1) can be

observed on the leaves, stems and spikes, but it is the leaves that are most often attacked. Generally, white pustules develop and produce a mass of spores with a powdery appearance. Gradually they grow, powdery mildew pustules darken to gray or brown [7, 8]. Over time, organs containing black spores (cleistothecia) are found to be embedded in the pustules of powdery mildew, usually towards the end of the season. All cereals can be attacked by powdery mildew. Several forms of the disease, however, are particular to specific cultures and do not cause cross-infection.

2.1.2 Life Cycle of Wheat Powdery Mildew

This wheat disease overwinters primarily as mycelium on volunteer cereals and crops sowed in autumn. Because the cleistothecia products in late summer resist to low temperatures and drought, this allows the fungus to survive in the absence of host. In the presence of high humidity, the cleistothecia releases ascospores produced by sexual reproduction, which can then cause autumnal infections [9]. It is estimated that cleistothecia is of secondary importance

Fig. 1 Symptoms of powdery mildew on wheat.

(a) Mature mildew pustules containing cleistothecia on the ears; (b) first symptoms of powdery mildew; (c) powdery mildew with cleistothecia on the leaf. (www.agro.basf.fr)

for the mycelium. In spring, with the temperature rises, the dormant mycelium begins to grow and spores are produced quickly. Germination occurs in a wide temperature range (from 5 °C to 30 °C), although 15 °C is the optimum temperature with greater than 95% relative humidity. Free water inhibits spore germination. In drought conditions, fresh spores can form after 7 d. At the end of the season, volunteer cereals and crops sowed before the season in turn may be contaminated, thus constituting the inoculum for the next crop.

2.1.3 Damage due to Wheat Powdery Mildew

The plots of winter wheat in late sowing are often particularly susceptible to the attack by powdery mildew, especially when cultures are developing rapidly in the spring. The use of large amounts of nitrogen fertilizer promotes disease and powdery mildew can be particularly severe in the dense plots. The visual appearance of the disease usually exceeds the harm that it can cause, especially in autumn and winter.

With susceptible varieties, yield losses can be high up to 20% and early control may be essential. However, the disease usually causes yield losses much smaller [10], and later attacks (after flowering) on flag leaf and spike rarely result in significant losses. Sometimes the disease appears simultaneously with other leaf diseases like rust or septoria, and it is very difficult to delimit the yield losses due to each one in field conditions [10].

2.2 *Fusarium* Head Blight

The disease affects wheat, barley, oats, rye, triticale and grasses. There are many species of the genus *Fusarium* spp. that affect cereals [11]. These fungi form a complex of diseases that infect grains, seedlings and mature plants. Generally, the pathogen—*Microdochium nidale* (formerly *Fusarium nivale*), transmitted by seeds, is also included in this group of fungi [12].

Microdochium nivale is the main pathogen group; it

causes seedling blight and thus causes the death of shoots and bleaching of spikes. Other species cause a variety of symptoms, including brown lesions at the base of the stems, which is often confined to the upper leaf sheath.

2.2.1 Symptoms of Wheat *Fusarium* Head Blight

The lesions caused by *Fusarium* often appear at the base of the stem and leaf sheath that coronal roots tear when they appear. This infection can then extend to the leaf sheath; the spread is manifested by the presence of long brown streaks at the base of the stem (Fig. 2). The most common symptom is dark brown lower nodes (Fig. 2). On older plants, *Fusarium* infection can cause a real rot and the base of the stem becomes brown and rotten, resulting in a pours and training of silver spikes [13]. This symptom is less common, although it can be observed during drought periods.

The infection often causes bleaching of all or part of the spike (Fig. 2). This symptom is observed when the spikes are infected in the early stages of flowering. The later infections can cause the infection of grains without significant bleaching of the spikes. Phase of spikes bleaching of that wheat disease can cause yield loss, but the main concern is the potential production of mycotoxins in grains. Mycotoxins are toxic to humans and animals [14-16]. Their concentration in the grain, flour and flour-based products intended for human and animal consumption is limited by European Union legislation.

2.2.2 Life Cycle

On plots of wheat, grains are the main source of *Fusarium*. However, the fungus can also survive in soil debris. In times of high humidity during flowering [17] and grain formation, the spores are dispersed by splashing the lower parts of plants, which causes bleaching and spikes infection by grains. During the same periods, infection by grains can seriously compromise the development of culture, unless the grains are treated with a fungicide directed against *Fusarium*.

Fig. 2 Symptoms of fusarium head blight of wheat.

(a) and (b) Bleaching of wheat (*Fusarium poae*); (c) fusarium head blight at the base of the stem; (d) coloring dew grains caused by fusarium head blight. (www.agro.basf.fr)

Fig. 3 Symptoms of wheat rust.

(a) Brown rust pustules on a leaf of wheat; (b) pustules on a leaf; (c) brown on stems, symptoms of black rust on wheat; (d) elongated pustules, yellow-orange color, arranged in a linear fashion between the veins on the upper leaf surface; (e) their particular disposition in long lines parallel to each other giving a drawn appearance to the leaves, symptoms of yellow rust on wheat. (www.agro.basf.fr)

2.2.3 Importance of Fusarium Head Blight

The symptoms of *Fusarium* infection are frequently observed in plots of wheat. When the weather is humid during flowering, the level of bleaching of spikes may be important. The impact of this symptom causes extensive losses worldwide [18]. The cases of severe rot are very rare and losses are usually minimal. The phase of propagation by grains can be particularly devastating. Antifungal therapy plays a major role in preventing the loss of wheat seedlings.

2.3 Wheat Rusts

2.3.1 Symptoms of Wheat Rusts

The pathogens causing wheat rust are: *Puccinia recondita* f. sp. *tritici*—brown rust agent [1], *Puccinia graminis* f. sp. *tritici*—black rust agent [19] and *Puccinia striiformis*—yellow rust agent [1].

The identification of rust is easy because they form characteristic pustules. Pustules correspond to a laceration of the epidermis, and the powder (orange, brown, brick red, dark brown or yellowish depending on the species) solely composes of spores that are easily carried by the wind. The species-specific rust symptoms are [2]:

Brown rust (leaf rust): small pustules size, circular or oval, orange or brownish. Preferably they appear on the upper leaf surface (Fig. 3a);

Black rust (stem rust): pustules longer than the brown rust, brick-red to dark maroon. It grows on the leaves and the stems (Figs. 3b and 3c). Black rust pustules produce spores on both sides of the leaf. Pustules on the stem are elongated vertically and have jagged pieces of torn epidermis along the sides. Reddish-brown powder spores are produced on both leaves and stems;

Yellow rust (stripe rust): yellowish pustules, aligned along the leaf veins in the form of streaks. Pustules also develop on the underside of leaves and spikes (Figs. 3d and 3e). It can be distinguished from other rusts by the dusty yellow urediniospores that produce in lesions and grow systemically in leaves.

The symptoms from overwintering infections occur on leaves closest to the ground and tend to cover the entire width of leaves rather than in stripes. The infection spreads to nearby plants and creates distinct “hot-spots” that can be seen from a distance by heading time.

2.3.2 Life Cycle of Wheat Rusts

The life cycles of cereal rusts are complex and often involve a primary host and alternative host. Among the three wheat rusts above, only the yellow rust has no alternative host [2]. In fact, *Puccinia graminis* and *Puccinia recondita* are macrocyclic and heteroecious. The alternate hosts of *Puccinia graminis* include *Berberis* spp. and *Mahonia* spp., and those of *Puccinia recondita* include species of *Thalictrum*, *Anchusa*, *Isopyrum* and *Clematis*. *Puccinia striiformis* has a microcyclic life cycle with no known alternate host. Where present, alternate hosts are important in disease epidemiology in providing local inoculum to initiate rust development in adjacent wheat crops and acting as a source of new pathotypes by hosting the sexual stage of the fungal life cycle [1].

The development of rust epidemics depends on the nature and quality of primary inoculums, varietal susceptibility, stage of wheat at the time of primary infection and climatic conditions. The source of inoculum is very important for the development of rust epidemic; there are two main sources of inoculum (made of uredospore): endogenous and exogenous.

The endogenous inoculum comes from a local conservation source (alternative host or volunteer wheat). Infections resulting from endogenous inoculum appear early at tillering. This gives rise later to the formation of foci of infection, whose characteristic is the presence of rust pustules on the lower leaves of the plant. Uredospores rusts can be carried by the wind for long distances and constitute an exogenous inoculum. The result of this inoculum pustules appears on the upper leaves of wheat. In this case, the disease is threatening when wet periods (rain, dew) are common. On the surface of leaves,

uredospores germinate in the presence of free water. Germination was done in 30 min at temperatures of 15-25 °C. The period between germination and sporulation is 8-20 d between 10 °C and 20 °C. On average, 3,000 uredospores are produced by pustule each day. Sporulation period is 5-25 d, depending on temperature and varieties. This explains the explosive nature of rusts, when conditions are favorable [2].

For yellow rust and in absence of alternative host, the pathogen must persist as vegetative cycle of uredospores to keep themselves in areas where it occurs. The first attack of the disease is often in the form of localized foci. Uredospores of yellow rust are very sensitive to ultraviolet rays, which reduce their viability in clear weather. During covered period, viable disseminations take place. Favorable days to contamination by yellow rust are characterized by an average temperature above 4 °C and night temperatures between 10 °C and 15 °C, with relative humidity above 80% for 18 h.

2.3.3 Importance of Wheat Rusts

The three species of rust above attack both bread wheat and durum wheat. About their relative importance, brown rust is the most widespread in its distribution, while the black rust is most devastating when it develops. Yellow rust is limited to the cool temperate climate and high altitude areas. The spatial and temporal distribution of three rusts is dictated by the different thermal requirements for development: brown rust develops at temperatures of 10-30 °C; the black rust is favored by wet conditions and high temperatures (15-35 °C); yellow rust develops between 2 °C and 15 °C [2].

3. Genetic Transformation of Wheat in the Fight against Fungal Disease

Wheat plants interact with their environment in various ways. They have to compete with their neighbours and endure the attack of fungal pathogen. Natural selection can improve the competitiveness and stress resistance [20]. Plants are equipped with the

natural plant defense system against fungi, which is provided by the proteinase inhibitors [21, 22]. However, there are no wild plants with resistance against all possible pathogens [23]. Many efforts have been made to improve the productivity of wheat crop under conditions of fungal stress. Although natural selection has favored mechanisms of adaptation and survival, multiplication by sexual crossing led the selection to increase economic performance of crops. But there is still a significant gap between yield under optimal conditions and stress conditions. Minimizing the “yield gap” and increasing yield stability in different conditions of stress are of strategic importance for the abundant production of wheat for the future [24]. Biotechnology by means of genetic transformation appears as an alternative choice for creating resistance varieties for fungal disease. The production of transgenic wheat will thus reduce the cost of production, increase yield and improve food quality.

Genetic engineering allows the expression of foreign genes from distant unrelated species as well as the modification of the usual pattern of expression of an already present gene. Among the various strategies to introduce fungal resistance by transgenic approach, strengthening the host plant defense by genetic manipulation holds tremendous potential [25]. Biochemical and structural responses against fungal attack include the reinforcement of plant cell wall and accumulation of phytoalexins with microbial toxicity, ribosome-inactivating proteins that inhibit protein synthesis, antimicrobial peptides and synthesis of other pathogenesis-related proteins [26-28]. Many plants also develop an increased resistance to subsequent pathogen infection in uninfected tissues. This systemic acquired resistance can be effective against viruses, bacteria and fungi, and is accompanied with the expression of a large set of genes termed pathogenesis-related genes [29, 30].

Pathogenesis-related genes probably have an important role in the defense response of plants

against fungal infections. The “antifungal protein” strategy involves the constitutive expression in transgenic plants of genes encoding proteins that have a fungitoxic or fungistatic capacity and therefore enhances resistance to fungal pathogens [31]. Liang et al. [32] reported that transgenic wheat plants with Aly alpha-fetoprotein (*AFP*) transgene showed the increased resistance to *Fusarium* sp. obtained using the above approach. Xing et al. [5] integrated a thaumatin-like protein (*Ta-Tlp*) (an important member of pathogenesis-related proteins) in wheat for resistance to powdery mildew, and all plants of initial generation (T0), first generation (T1) and second generation (T2) were resistant to wheat powdery mildew by delaying disease development [5].

In addition, other gene, like the wheat *Lr34* gene, is one of the few cloned and durable resistance genes in plants. It encodes an ATP-binding cassette G subfamily (ABCG) transporter and has been a source of resistance against biotrophic pathogens, such as leaf rust, for over 100 years [33]. As the endogenous *Lr34* confers quantitative resistance, Risk et al. [33] determined the effects of transgenic *Lr34* with specific reference to how expression levels affect resistance. Transgenic *Lr34* wheat lines were made in two different, susceptible genetic backgrounds. They found that the introduction of the *Lr34* resistance allele was sufficient to provide comparable levels of leaf rust resistance as the endogenous *Lr34* gene. As with the endogenous gene, they observed the resistance in seedlings after cold treatment and in flag leaves of adult plants as well as *Lr34*-associated leaf tip necrosis. Makandar [18] showed that the *Arabidopsis thaliana NPR1* gene (*AtNPR1*), which regulates the activation of systemic acquired resistance, when expressed in the fusarium head blight-susceptible wheat cv. Bobwhite, confers a heritable, type II resistance to fusarium head blight caused by *Fusarium*. It is known that the contamination of food with *Fusarium* producing trichothecene mycotoxin deoxynivalenol is a great

health risk for humans and animals, because trichothecenes are potent cytotoxins of eukaryotic cells. In this context, Di et al. [34] have demonstrated the expression of an N-terminal fragment of yeast L3 (*L3Δ*) in wheat, which showed the reduction in disease severity and improved level of deoxynivalenol in transgenic wheat kernel as compared to non-transgenic wheat plants.

4. Conclusions

Fungal pathogens are a major cause of yield loss in wheat and its resistance to fungal pathogens is fundamental to global food security. After the identification of disease-specific symptoms, it will be logical to think about treatment. To reduce crop loss, the wheat production is dependent on new and improved varieties with resistance to the rapidly evolving wheat fungal diseases. The advent of genetic engineering by genetic transformation has enabled plant biologists to fight against fungal by integrating stable transgene known to confer resistance to powdery mildew, fusarium head blight and rust. Although there is still no transgenic wheat approved for commercial production in the world, but it is inevitable that it will be approved within the next few years; genetically-modified wheat will undoubtedly add value to many stages of the production and utilization chain.

Acknowledgments

The authors would like to thank the Morocco Ministry of Agriculture and the Morocco Ministry of Higher Education for supporting this work.

References

- [1] McIntosh, R. A., Wellings, C. R., and Park, R. F. 1995. *Wheat Rusts: An Atlas of Resistance Genes*. Australia: CSIRO Publications, 205.
- [2] Ezzahiri, B. 2001. “Diseases of Wheat: Identification, Development Factors and Methods.” Technology Transfer in Agriculture, Bulletin Mensuel d’Information et de Liaison du PNTTA. Accessed July 13, 2013. <http://www.agrimaroc.net/77.pdf>. (in French)

- [3] Scheider, N., Guo, J. R., Verre, J. A., and Beyer, M. 2009. "Assessing the Intensity of *Fusarium*-Damage in Wheat: A Comparison of Selected Disease Parameters during Disease Development and the Role of Fungicides." *J. Plant Dis. Prot.* 116 (3): 118-23.
- [4] Meyer, J. B., Song-Wilson, Y., Foetzki, A., Luginbühl, C., Winzeler, M., Kneubühler, Y., Matasci, C., Mascher-Frutschi, F., Kalinina, O., Boller, T., Keel, C., and Maurhofer, M. 2013. "Does Wheat Genetically Modified for Disease Resistance Affect Root-Colonizing Pseudomonads and Arbuscular Mycorrhizal Fungi?" *PLOS One* 8 (1): e53825.
- [5] Xing, L. P., Wang, H. Z., Jiang, Z. N., Ni, J. L., Cao, A. Z., Yu, L., and Chen, P. D. 2008. "Transformation of Wheat Thaumatin-Like Protein Gene and Analysis of Reactions to Powdery Mildew and Fusarium Head Blight in Transgenic Plants." *Acta Agron. Sini.* 34 (3): 349-54.
- [6] Miroshnichenko, D., Filippov, M., and Dolgov, S. 2014. "Genetic Transformation of Russian Wheat Varieties for the Biotic and Abiotic Stress Resistance." In *Proceedings of the 4th International Crop Science Congress*, 613.
- [7] Wiese, M. V. 1987. *Compendium of Wheat Diseases*, 2nd ed.. St. Paul, MN, USA: APS Press, 124.
- [8] Daamen, R. A. 1989. "Assessment of the Profile of Powdery Mildew and Its Damage Function at Low Disease Intensities in Field Experiments with Winter Wheat." *Neth. J. Plant Pathol.* 95 (2): 85-105.
- [9] Parks, R., Carbone, I., Murphy, J. P., Marshall, D., and Cowger, C. 2008. "Virulence Structure of the Eastern U.S. Wheat Powdery Mildew Population." *Plant Dis.* 92 (7): 1074-82.
- [10] Tomás, A., and Solís, I. 2000. "Effects of Powdery Mildew (*Blumeria graminis*) Severity on Durum Wheat Cultivars." In *Durum Wheat Improvement in the Mediterranean Region: New Challenges*, edited by Royo, C., Nachit, M., Di-Fonzo, N., and Araus, J. L. Zaragoza: CIHEAM, 417-9.
- [11] Popovski, S., and Celar, F. A. 2013. "The Impact of Environmental Factors on the Infection of Cereals with *Fusarium* Species and Mycotoxin Production—A Review." *Acta Agr. Sloven.* 101 (1): 105-16.
- [12] Brennan, J. M., Fagan, B., Van Maanen, A., Cooke, B. M., and Doohan, F. M. 2003. "Studies on *in Vitro* Growth and Pathogenicity of *Fusarium* Fungi." *Eur. J. Plant Pathol.* 109 (6): 577-87.
- [13] Parry, D. W., Jenkinson, P., and McLeod, L. 1995. "Fusarium Ear Blight (Scab) in Small Grain Cereals—A Review." *Plant Pathol.* 44 (2): 207-38.
- [14] D'Mello, J. P. F., Placinta, C. M., and Macdonald, A. M. C. 1999. "Fusarium Mycotoxins: A Review of Global Implications for Animal Health, Welfare and Productivity." *Ani. Feed Sci. Tech.* 80 (3-4): 183-205.
- [15] Placinta, C. M., D'Mello, J. P. F., and Macdonald, A. M. C. 1999. "A Review of Worldwide Contamination of Cereal Grains and Animal Feed with *Fusarium* Mycotoxins." *Ani. Feed Sci. Tech.* 78 (1-2): 21-37.
- [16] Golinski, P., Waskiewicz, A., Wisniewska, H., Kiecana, I., Mielniczuk, E., Gromadzka, K., Kostecki, M., Bocianowski, J., and Rymaniak, E. 2010. "Reaction of Winter Wheat (*Triticum aestivum* L.) Cultivars to Infection with *Fusarium* spp.: Mycotoxin Contamination in Grain and Chaff." *Food Addit. Contam. Part A* 27 (7): 1015-24.
- [17] Muthomi, J. W., and Mutitu, E. W. 2003. "Occurrence of Mycotoxin Producing *Fusarium* Species and Other Fungi on Wheat Kernels Harvested in Selected Districts of Kenya." *Afri. Crop Sci. Con. Proc.* 6: 335-9.
- [18] Makandar, R., Essig, J. S., Schapaugh, M. A., Trick, H. N., and Shah, J. 2006. "Genetically Engineered Resistance to Fusarium Head Blight in Wheat by Expression of *Arabidopsis NPR1*." *Mol. Plant Mic. Inter.* 19 (2): 123-9.
- [19] Schumann, G. L., and Leonard, K. J. 2000. "Stem Rust of Wheat (Black Rust)." The Plant Health Instructor. Accessed July 13, 2013. <http://www.apsnet.org/edcenter/intropp/lessons/fungi/Basidiomycetes/Pages/StemRust.aspx>.
- [20] Zeller, S. L., Kalinina, O., and Schmid, B. 2013. "Costs of Resistance to Fungal Pathogens in Genetically Modified Wheat." *J. Plant Ecol.* 6 (1): 92-100.
- [21] Larry, L. M., and Richard, E. S. 2002. "Lectins and Protease Inhibitors as Plant Defenses against Insects." *J. Agr. Food Chem.* 50 (22): 6605-11.
- [22] Kim, J. Y., Park, S. C., Hwang, I., Cheong, H., Nah, J. W., Hahm, K. S., and Park, Y. 2009. "Protease Inhibitors from Plants with Antimicrobial Activity." *Int. J. Mol. Sci.* 10 (6): 2860-72.
- [23] Bergelson, J., and Purrington, C. B. 1996. "Surveying Patterns in the Cost of Resistance in Plants." *Am. Nat.* 148 (3): 536-58.
- [24] Patnaik, D., and Khurana, P. 2001. "Wheat Biotechnology: A Minireview." *Electron. J. Biotech.* 4 (2): 74-102.
- [25] Cattivelli, L., Rizza, F., Badeck, F. W., Mazzucotelli, E., Mastrangelo, A. M., Francia, E., Marè, C., Tondelli, A., and Stanca, A. M. 2008. "Drought Tolerance Improvement in Crop Plants: An Integrated View from Breeding to Genomics." *Field Crop Res.* 105 (1-2): 1-14.
- [26] Bowles, D. J. 1990. "Defense-Related Proteins in Higher Plants." *Annu. Rev. Biochem.* 59: 873-907.
- [27] Yang, Y., Shah, J., and Klessig, D. F. 1997. "Signal Perception and Transduction in Plant Defense Responses." *Genes Dev.* 11 (13): 1621-39.
- [28] Engel, K. H., Frenzel, T., and Miller, A. 2002. "Current

- and Future Benefits from the Use of GM Technology in Food Production.” *Toxicol. Lett.* 127: 329-36.
- [29] Van Loon, L. C., Gerritsen, Y. A. M., and Ritter, C. E. 1987. “Identification, Purification and Characterization of Pathogenesis-Related Proteins from Virus Infected Samsun NN Tobacco Leaves.” *Plant Mol. Biol.* 9 (6): 593-609.
- [30] Ward, E. R., Uknes, S. J., Williams, S. C., Dincher, S. S., Wiederhold, D. L., Alexander, D. C., Ahl-Goy, P., Metraux, J. P., and Ryals, J. A. 1991. “Coordinate Gene Activity in Response to Agents that Induce Systemic Acquired Resistance.” *Plant Cell* 3 (10): 1085-94.
- [31] Melchers, L. S., and Stuiiver, M. H. 2000. “Novel Genes for Disease-Resistance Breeding.” *Curr. Opin. Plant Biol.* 3 (2): 147-52.
- [32] Liang, J., Wu, Y., Rosenberger, C., Hakimi, S., Castro, S., and Berg, J. 1998. “AFP Genes Confer Disease Resistance to Transgenic Potato and Wheat Plants.” Presented at the 5th International Workshop on Pathogenesis-Related Proteins in Plants—Signalling Pathways and Biological Activities, Aussois, France.
- [33] Risk, J. M., Selter, L. L., Krattinger, S. G., Viccars, L. A., Richardson, T. M., Buesing, G., Herren, G., Lagudah, E. S., and Keller, B. 2012. “Functional Variability of the Lr34 Durable Resistance Gene in Transgenic Wheat.” *Plant Biotech. J.* 10 (4): 477-87.
- [34] Di, R., Blechl, A., Dill-Macky, R., Tortora, A., and Tumer, N. E. 2010. “Expression of a Truncated Form of Yeast Ribosomal Protein L3 in Transgenic Wheat Improves Resistance to Fusarium Head Blight.” *Plant Sci.* 178 (4): 374-80.