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Plant biotechnology in agriculture

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Abstract

Knowledge on plant genomes has progressed during the past few years. Two plant genomes, those of *Arabidopsis thaliana* and rice, have been sequenced. Our present knowledge of synteny also indicates that, despite plasticity contributing to the diversity of the plant genomes, the organization of genes is conserved within large sections of chromosomes. In parallel, novel plant transformation systems have been proposed, notably with regard to plastid transformation and the removal of selectable marker genes in transgenic plants. Furthermore, a number of recent works considerably widen the potential of plant biotechnology.

Keywords: Biotechnology; Transgenesis; Plants

1. Introduction

Prior to agriculture, humans lived as nomadic hunters and could survive solely on wild plant and animal resources. Noticing the immense wealth of the plant and animal kingdoms, their successful efforts to domesticate the wild species launched agriculture. Until very recently, plant breeding still relied solely on the accumulated experience of generations of farmers and breeders, that is, on sexual transfer of genes between plant species. However, recent developments in plant molecular biology and genomics now give us access to the knowledge and understanding of plant genomes and even the possibility of modifying them. Plant geneticists have adopted *Arabidopsis thaliana* as a model organism some years ago because of its small diploid genome (the *Arabidopsis* genome, at about 120 Mb, is amongst the smallest known plant genomes), low repetitive DNA content, and rapid reproductive rate. Now, the complete sequence of the genome of this plant is known, which will lead to the identification of all its 26,000 genes [1]. Based on this success, the sequencing of various cultivated plant genomes is now well underway (e.g. rice). The rice genome sequence provides a foundation for the improvement of cereals, our most important crops [2,3]. Most importantly, our present knowledge of synteny indicates that, despite plasticity contributing to the diversity of the plant genomes, the organization of genes is conserved within large sections of chromosomes [4–8]. This validates a posteriori the considerable efforts made on model species. Such progress has encouraged a massive surge in plant biotechnology, which is currently changing our vision of crop production and protection. Indeed, this technological progress enables us to insert useful genes into cultivated plants at an incomparably fast rate and, doubtless, in a much more precise manner than with conventional genetic methods.

2. Plant transformation

Genetic engineering techniques now allow us to transfer the genes of one species over to another species. Indeed, the intended uses aim to introduce new characters into an organism that otherwise would not have acquired them. These techniques can be applied generally to all living species: bacteria, fungi, viruses, animals, and plants. After undergoing genetic engineering, the organisms are referred to as genetically modified organisms (GMOs) to indicate that they

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are organisms that have had their genetic profile altered in a laboratory. In the case of plants, one of the elements of the current social debate is based on the fact that these new transplanted characters can originate from a species totally unrelated to the receiving plant.

2.1. Transformation techniques and search for new selectable markers

The deliberate incorporation of genes in the nuclear genome of plants can be carried out using different techniques. One transfer method, known as the biolistic technique, consists in the gene of interest being coated onto either gold or tungsten particles [9]. The charged particles are then shot into plant cells using a gene-gun (the necessary criteria for the choice of these cells or plant tissues are that they be apt for transformation and permit later regeneration of a whole plant). After this operation, some cells are transformed; that is to say, they have integrated the gene of interest into their genome. The other major gene transfer technique uses the soil bacterium *Agrobacterium tumefaciens*, which has the natural property of inducing tumors (crown gall) in certain plants by transmitting a plasmid (T-DNA). This naturally occurring plant transformation results in the integration of virulent bacterial genes into the plant genome. Thanks to more than 20 years of intense research, the genes responsible for provoking the tumors have been eliminated from this plasmid and replaced by selected genes that can be inserted into the plant cells genome [10–15]. In every case, these transformation systems lead to random integration of the gene of interest in the receiving genome.

The transferred constructs generally contain a promoter element that allows the regulation of transgene expression, either quantitatively or in a specific manner. They also contain a selectable marker gene that codes for an easily recognizable characteristic, for example, a herbicide or antibiotic resistance gene. Only the rare transformed plants will survive on media containing the corresponding herbicide or antibiotic, thus providing a means for selection. To address the issue of the risk of spreading antibiotic/herbicide-tolerant genes in the environment, other systems have recently been developed. One system differs from conventional selection techniques as it is based on supplementing the transgenic cells with a metabolic advantage rather than killing the non-transgenic cells. For example, a selection technique is based on the use of mannose and the *pmi* bacterial gene encoding the enzyme phosphomannose isomerase. In plants, mannose is transformed into mannose-6P, which strongly inhibits growth. Only in those transgenic plants that acquire the *pmi* gene can mannose-6P be transformed further into fructose-6P, thereby allowing their survival on mannose rich culture media [16]. Another improvement in plant transformation is based upon the utilization of genes promoting endogenous hormone production under the control of chemical stimulants. One such system uses the isopentenyltransferase (*ipt*) gene from the Ti plasmid of *A. tumefaciens* to increase cytokinin levels. In the presence of the chemical inducer, transformed plant cells can be easily recognized by a shooty phenotype. When transformed shoots are transferred to an inducer-free medium, whole fertile plants can be recovered with high efficiency [17].

2.2. Engineering the nuclear genome without antibiotic resistance genes

Toward the need of limiting the presence of unnecessary genes in the plant products, there have been several attempts to remove transferred DNA in transgenic plants. One strategy is based on the use of the Cre-*lox* site-specific recombination system, which usually involves two steps. The first consists in transforming plants with a plasmid containing the transgene of interest and a resistance gene bordered by two *lox* sites. The second consists in a transformation with another plasmid containing the *Cre* gene encoding the recombinase. Transformed plants are crossed, and a segregation analysis of the F1 progeny is performed, allowing the identification of plants that only harbor the transgene of interest but not the selectable marker [18,19]. Overall, these two-step transformation processes are much more laborious than conventional transformation methods. Simplified single-step procedures have been recently published [20,21].

2.3. Engineering the plastid genome

Plastidial DNA transformation in certain species (tobacco in particular) is now under control. There are several advantages to developing this new technique. These include (a) the transfer of the desired genes solely via the female line (in most plant species, the pollen does not contain any plastids), thereby limiting the contamination of wild plants with transgenes carried on by pollen flow; (b) very high levels of transgene expression in genetically engineered plants (indeed, each plant cell contains many hundreds of plastids; furthermore, gene silencing, frequently observed in nuclear transgenic plants, has not been observed in genetically engineered chloroplasts); (c) targeted homologous recombination into the plastid genome [22,23]. As for the nuclear transgenic plants, several strategies have been developed for engineering chloroplasts that are free of antibiotic resistance markers [23,24].

Beyond this current stage, where the feasibility of plant transgenesis and its application in various fields (see below) has been proven, it is important to note that transgenesis is not as yet routinely applicable to all plant species. For example, the transformation of certain leguminous plants is still difficult to achieve [25].

3. Fields of application

Plants constitute the main food resource for animals and humans. This is why the foremost mission of agriculture is to produce plants in sufficient quantities to be able to respond to the absolute necessity of feeding the world. Today, this problem has become acute in the face of the demographic explosion, the erosion of arable land, and the development of intensive farming causing environmental damage. Genetic engineering of plants can offer good solutions to these problems [26–28].

For the past 10,000 years, man has endeavored to domesticate plants, selecting seed of preferred forms and culling out seed of undesirable types to produce each subsequent generation [29,30]. Plant breeding has brought about a spectacular increase in yields (e.g. an increase of between five and 10 times over the last century for cereal crops). Several traits have been considerably improved, for example, crop establishment, plant growth, plant resistance to pathogens and pests, and grain yield. This activity of domestication has, however, led to the breeding of species that are incapable of surviving in the wild and has attenuated, and even eliminated, certain mechanisms that the wild plants had developed to ensure their survival. This is the case, for example, with dormancy mechanisms, one of the multiple regulatory systems involved in seed germination. These dormancy periods benefit wild species because they allow seeds to remain viable while the conditions necessary for germination are unfulfilled, but this same factor poses a problem when it comes to cultivated species. In general, most cultivated plants have lost the typical invasiveness properties shown by wild plants, and their survival now solely depends on man, notably as regards the addition of chemical fertilizers and plant protection products.

Following on from this long tradition of plant breeding, the aims of plant biotechnology are manifold. Without being exhaustive, this presentation illustrates some applications that have the same goals as conventional breeding methods and others that are specific to transgenesis, being unattainable using a conventional approach.

3.1. Controlling plant development and yield

Important factors targeted by biotechnological approaches are dispersion and seed dormancy. For example, in the oilseed rape, the seeds that fall to the ground during dehiscence represent a loss of almost 20% of the harvest for the farmer. To address this problem, the genes responsible for silique opening during dehiscence have been identified in *Arabidopsis thaliana*[31]. Moreover, recent work has shown that it is possible to genetically control dormancy, with regard both to hormone biosynthesis (abscissic acid, gibberellins) and to transcription factors involved in the phenomenon [32]. Biotechnology can also play an important part in improving crop yield. There are many examples documenting this possibility. A recent one shows that enhanced activity of ADP-glucose pyrophosphorylase (an enzyme that plays a key role in regulating starch biosynthesis in cereal seeds) in wheat endosperm increases seed yield by as much as 40% [33].

There are many examples concerning herbicide, fungal pathogen, virus, and insect resistant plants, because large agrochemical companies are particularly active in this sector. Nonetheless, the progress made has not all been as regards technique. Fundamental knowledge of plants has, indeed, progressed enormously from these application-oriented studies. Let us underline, for example, the characterization of biosynthetic pathways for aromatic amino acids in plants (tryptophane, tyrosine, phenylalanine; the inhibitor for 5-*enol*pyruvoylshikimate-3-phosphate synthase, glyphosate, is the active ingredient in the commercial herbicide Roundup Ready), or that for the branched-chain amino acids (valine, isoleucine, leucine; the inhibitors for acetolactate synthase, sulfonylureas and imidazolinones, are among commercialized herbicides, the most active and specific acting at dose rates of a few grams per hectare) [34].

The main arguments put forward for the intensive introduction of these transgenic plants in agriculture are the following. (a) The existence of farmer demand for techniques facilitating their work. Indeed, appropriate chemical treatment carried out during sowing can be enough to eradicate weeds. Moreover, these same plants, made pest resistant, would not need conventional chemical control. (b) The introduction in a susceptible plant of a tolerance to a selective herbicide would allow this particular herbicide's market to be broadened. In addition, plant biotechnology could enable the development of herbicides that would otherwise never have been developed for lack of selectivity. (c) The development of such plants could considerably lessen production losses in cases with no treatment. (d) The use of these plants could bring about a major reduction in chemical treatments used on crops or encourage the use of products less toxic for the environment. Although a precise figure has still not been put to the economic and ecological benefits that could be generated by these new technologies, it should be pointed out that recent estimates in the US show a considerable reduction in the use of chemical insecticides, around 1000 tonnes, since the introduction of transgenic cotton that expresses the insecticidal Bt toxin derived from the soil bacterium *Bacillus thuringiensis* [35].

The success of current plant biotechnology is based on the hypothesis that resistance to herbicides, insects, and viruses can be obtained by inserting a limited number of genes into cultivated plants. This is well demonstrated by varieties of transgenic plants that are resistant to insects through expression of a single protein, the Bt toxin. In the same way, a resistance to insects can be obtained in cultivated plants through expression of protease inhibitors or α-amylase [36]. In the case of herbicides, this idea has been fuelled principally by the observation that the "simple" overexpression of target enzymes (whether natural or mutated) of these herbicides effectively renders transgenic lines tolerant. It is impor-

tant to note here that although commercial herbicides are extremely efficient, they are, nevertheless, often lacking in specificity, and therefore, their commercialization is largely dependent on the introduction of tolerance in the most widely grown crop species. This strategy favors de facto the development and commercialization of a restricted number of plant protection products. The following heavily debated problems could ensue: (a) natural resistance engendered by intensive use of a few herbicides (one solution would be to alternate crops and chemical treatments); (b) the modification of ecosystems, giving rise to the development of resistant pests (one solution would be the cultivation of non-transgenic crops amongst transgenic crops, serving as attractants, or refuge, for pests); (c) the dissemination of transgenes (in the face of this important question, we have mentioned above the possibility of introducing these genetic modifications not into the nuclear genome, but into that of the plastid. We have also mentioned the emergence of new marker genes, and of new strategies to remove the marker genes in both nuclear and plastid genomes, thus avoiding the questionable use of antibiotic and herbicide resistance genes).

3.2. Improving the tolerance of plants to biotic stresses

Environmental factors are essential components in crop yields. The introduction of resistance to heavy metals, salt, cold, and drought into crop plants has become a topic of major economic interest for agriculture. Genetically engineered drought- and salt-tolerant plants could provide an avenue to the reclamation of farmlands lost to agriculture because of salinity and a lack of rainfall. In the case of drought, we are beginning to understand certain extremely complex mechanisms through which seeds from orthodox plants acquire tolerance to desiccation during their final maturation period, when the seed becomes quiescent and its metabolism stops. Reviviscent plants, capable of supporting extreme hydrous stress, provide another model. Some of the genes associated with the acquisition of this tolerance to drought have been isolated and characterized. Based on this knowledge, there are several examples showing the feasibility of improving tolerance of plants to biotic stress by genetic engineering [37–39]. Finally, it is interesting to note that the genetic engineering of the signaling pathway implicating the gibberellin hormones allows the creation of plants that are more resistant to bad weather (wind, rain) [40].

The issue of the dissemination of such genes into the environment, however, is increasingly more important than in the case of those genes that confer a resistance to herbicides. This is because the genes for tolerance to biotic stresses could confer a real selective advantage to weeds, rendering them even more harmful.

3.3. The plant as a factory to produce useful molecules

A particularly fruitful area of research for the current interests of plant biotechnology concerns the improvement of the quality of plant products. It is clear that one can now alter the principal biosynthetic routes of the higher plants almost “at will” in order to make them synthesize new types of fatty acids, starch, and proteins. Their metabolite content, which is indispensable to animal and human nutrition (e.g. vitamins, essential amino acids), may also be modified. This is also the case for metabolites posing a problem to developing industrial applications (e.g. lignin, the principal constituent in wood, which poses problems in the paper industry). In the short and medium term, the potential for this area appears clearly to be much larger than those of resistance to herbicides or to insects that have been implemented until now. Although this is not an exhaustive list, this field of application is concerned with remodeling the constituents of oil and seed starch for not only nutritional and pharmaceutical but also industrial means (such as in detergents, lubricants, inks, polymers, cosmetics, plasticizers, biofuels, depolluting agents, etc.). It is also concerned with remodeling components essential to animal and human health (essential amino acids, vitamins) [34,41], plant metabolism rerouting for biodegradable plastic manufacture, or therapeutic proteins and enzymes (e.g. enzymes involved in lipid metabolism, vaccinal plants, monoclonal antibodies, etc.) [42–49].

This can be exemplified with some studies. The first exploits the specificity of plant metabolism and relates to the synthesis of essential amino acids (particularly lysine, threonine, and methionine) and of principal vitamins. It would appear that the levels of biosynthesis in these components are well adapted to plant needs (particularly those of the main field crops). On the other hand, they are not adapted to completely satisfy animal and human nutrition requirements. Therefore, nutritional deficiencies ensue, particularly severe in the populations of developing countries, which are more dependent on a supply of plants in their diet than are those of industrialized countries. To compensate for these deficiencies, plant biotechnology is aimed at improving the levels of those components in the field crops: for example, lysine and threonine in cereals, methionine in leguminous plants, and vitamins A and E in crucifers and rice, the latter constituting the staple food for a third of the world’s population. In certain cases, such research is producing promising responses (increase in the level of methionine and vitamins, for example) [50–52]. Increasing provitamin A content in rice is a major concern to prevent blindness in children. Rice endosperm does not contain any provitamin A. Transgenic rice containing four genes isolated from *Narcissus* and *Erwinia* has been obtained [51]. Some of the stable transgenic lines accumulate high amounts of provitamin A, giving the endosperm a yellow color; hence the name golden rice. On the other hand, the problem of controlling levels of lysine in cereals has not yet been resolved. However, research in this area has significantly contributed to enhancing knowledge of the mecha-

nisms that regulate the homeostasis of essential amino acids in plants (particularly concerning the role of catabolism), and so we can reasonably hope that, on its completion, the technological barriers will be lifted.

4. Conclusion

A number of recent works considerably widen the potential of plant biotechnology. In addition, transformation and breeding techniques have evolved, allowing us to respond, at least in part, to criticisms raised by the use of transgenic plants in agriculture. The development of these plant biotechnologies even now provokes many reactions. Among these are reactions to fields of application, modes of use, increased competence in agricultural procedures by agrochemical companies (appropriation of living organisms, modification of agricultural practices), and the reality and eventual consequences of transferring these techniques to developing countries [53]. Despite this, undeniable progress has been recorded on a scientific level, allowing, thanks to these technologies, useful modification of plants. This allows us to envisage the feasibility of improving the nutritional conditions for animals and humans (such as yield increase, correction of nutritional deficiency, elimination of antinutritional components, vitamin intake) and health (protein and enzymes manufacture, vaccinal plants), to encourage the arrival of multiple new industry-oriented products and to produce crops that work in greater harmony with the environment in terms of their water requirements, pesticides, and fertilizers. These techniques, although still not quite perfected, are now in place. Considerable advances have been made in our knowledge of the biochemistry of plants and their genome. These appear to be technically compatible with a cautious and reasoned introduction, case by case, of transgenic plants into modern agriculture. The finalization of new breeding methods, not based on the use of antibiotics or herbicides, and the transformation of plastids provide examples that demonstrate a positive influence on the social debate surrounding the development of research in this area. All that remains, and there is consensus on this point, is to thoroughly evaluate the environmental impact of these transgenic crops. As stressed by Potrykus [52], we need more examples of the "golden rice" type.

References

[1] The Arabidopsis Genome Initiative, Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*, Nature 408 (2000) 796–815.

[2] S.A. Goff, D. Ricke, T.H. Lan, G. Presting, R. Wang, M. Dunn, et al., A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*), Science 296 (2002) 92–100.

[3] J. Yu, S. Hu, J. Wang, G.K. Wong, S. Li, B. Liu, et al., A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*), Science 296 (2002) 79–92.

[4] H.M. Ku, T. Vision, J. Liu, S.D. Tanksley, Comparing sequenced segments of the tomato and *Arabidopsis* genomes: large-scale duplication followed by selective gene loss creates a network of synteny, Proc. Natl. Acad. Sci. USA 97 (2000) 9121–9126.

[5] D. Grant, P. Cregan, R.C. Shoemaker, Genome organization in dicots: genome duplication in *Arabidopsis* and synteny between soybean and *Arabidopsis*, Proc. Natl. Acad. Sci. USA 97 (2000) 4168–4173.

[6] R. Schmidt, Synteny: recent advances and future prospects, Curr. Opin. Plant. Biol 3 (2000) 97–102.

[7] S. Barnes, Comparing *Arabidopsis* to other flowering plants, Curr. Opin. Plant. Biol. 5 (2002) 128–134.

[8] J. Salse, B. Piegu, R. Cooke, M. Delseny, Synteny between *Arabidopsis thaliana* and rice at the genome level: a tool to identify conservation in the ongoing rice genome sequencing project, Nucl. Acids Res. 30 (2002) 2316–2328.

[9] T.M. Klein, R. Arentzen, P.A. Lewis, S. Fitzpatrick-McElligott, Transformation of microbes, plants and animals by particle bombardment, Bio/Technoloy 10 (1992) 286–291.

[10] M.D. Chilton, M.H. Drummond, D.J. Merlo, D. Sciaky, A.L. Montoya, M.P. Gordon, E.W. Nester, Stable incorporation of plasmid DNA into higher plant cells: the molecular basis of crown gall tumorigenesis, Cell 11 (1977) 263–271.

[11] P. Zambryski, H. Joss, C. Genetello, J. Leemans, M. Van Montagu, J. Schell, Ti plasmid vector for the introduction of DNA into plant cells without alteration of their normal regeneration capacity, EMBO J. 2 (1983) 2143–2150.

[12] Bevan, *Agrobacterium* vectors for plant transformation, Nucl. Acids Res. 12 (1984) 8711–8721.

[13] J. Schell, Transgenic plants as tools to study the molecular organization of plant genes, Science 237 (1987) 1176–1183.

[14] P.J.J. Hooykaas, R. Schilperoort, *Agrobacterium* and plant genetic engineering, Plant Mol. Biol. 19 (1992) 15–38.

[15] N. Betchold, J. Ellis, G. Pelletier, Transformation in planta de plantes adultes d'*Arabidopsis thaliana* par infiltration d'*Agrobacterium*, C. R. Acad. Sci. Paris 316 (1993) 1194–1199.

[16] M. Joersbo, Advances in the selection of transgenic plants using nonantibiotic marker genes, Physiol. Plant 111 (2001) 269–272.

[17] T. Kunkel, Q.W. Niu, Y.S. Chan, N.H. Chua, Inducible isopentenyl transferase as a high-efficiency marker for plant transformation, Nat. Biotechnol. 17 (1999) 916–919.

[18] D.W. Ow, Recombinase-directed chromosome engineering in plants, Curr. Opin. Biotechnol. 2 (1996) 181–186.

[19] D.W. Ow, Recombinase-directed plant transformation for the post-genomic era, Plant Mol. Biol. 48 (2002) 183–200.

[20] S. Endo, K. Sugita, M. Sakai, H. Tanaka, H. Ebinuma, Single-step transformation for generating marker-free transgenic rice using the *ipt*-type MAT vector system, Plant J. 30 (2002) 115–122.

[21] P.D. Hare, N.H. Chua, Excision of selectable marker genes from transgenic plants, Nat. Biotechnol. 20 (2002) 575–580.

[22] P. Maliga, P.J. Nixon, Judging the homoplastomic state of plastid transformants, Trends Plant Sci. 3 (1998) 376–377.

[23] H. Daniell, M.S. Khan, L. Allison, Milestones in chloroplast genetic engineering: an environmentally friendly era in biotechnology, Trends Plant Sci. 7 (2002) 84–91.

[24] S. Corneille, K. Lutz, Z. Svab, P. Maliga, Efficient elimination of selectable marker genes from the plastid genome by the CRE-*lox* site-specific recombination system, Plant J. 27 (2001) 171–178.

[25] R.G. Birch, Plant transformation: problems and strategies for practical application, Annu. Rev. Plant Physiol. Plant. Mol. Biol. 48 (1997) 297–326.

[26] M.J. Chrispeels, Biotechnology and the poor, Plant Physiol. 124 (2000) 3–6.

[27] L.R. Herrera-Estrella, Genetically modified crops and developing countries, Plant Physiol. 124 (2000) 923–926.

[28] A.J. Trewavas, The population/biodiversity paradox. Agriculture efficiency to save wilderness, Plant Physiol. 125 (2001) 174–179.

[29] R.L. Wang, A. Stec, J. Hey, J. Lukens, J. Doebley, The limits of selection during maize domestication, Nature 398 (1999) 236–239.
[30] C.S. Prakash, The genetically modified crop debate in the context of agricultural evolution, Plant Physiol. 126 (2001) 8–15.
[31] S.J. Liljegren, G.S. Ditta, Y. Eshed, B. Savidge, B.L. Bowman, M.F. Yanofsky, Shatterproof MADS-box genes control seed dispersal in *Arabidopsis*, Nature 404 (2000) 766–770.
[32] A. Frey, C. Audran, E. Marin, B. Sotta, A. Marion-Poll, Engineering seed dormancy by the modification of zeaxanthin epoxidase gene expression, Plant Mol. Biol. 39 (1999) 1267–1274.
[33] E.D. Smidansky, M. Clancy, F.D. Meyer, S.P. Lanning, N.K. Blake, L.E. Talbert, M.J. Giroux, Enhanced ADP-glucose pyrophosphorylase activity in wheat endosperm increases seed yield, Proc. Natl. Acad. Sci. USA 99 (2002) 1724–1729.
[34] J.F. Morot-Gaudry, D. Job, P.J. Lea, Amino acid metabolism, in: P.J. Lea, J.F. Morot-Gaudry (Eds.), Plant Nitrogen, INRA editions, Springer, New York, 2001, pp. 167–211.
[35] B.J. Miflin, Crop biotechnology. Where now?, Plant Physiol. 123 (2000) 17–27.
[36] O.L. Franco, D.J. Rigden, F.R. Melo, M.F. Grossi-de Sá, Plant α-amylase inhibitors and their interactions with insect α-amylases. Structure, function and potential for crop protection, Eur. J. Biochem. 269 (2002) 397–412.
[37] D. Xu, X. Duan, B. Wang, B. Hong, T.H.D. Ho, R. Wu, Expression of a late embryogenesis abundant protein gene, *HVA1*, from barley confers tolerance to water deficit and salt stress in transgenic rice, Plant Physiol. 110 (1996) 249–257.
[38] R.A. Gaxiola, J. Li, S. Undurraga, L.M. Dang, G.J. Allen, S.L. Alper, G.R. Fink, Drought- and salt-tolerant plants result from overexpression of the AVP1 H^+-pump, Proc. Natl. Acad. Sci. USA 98 (2001) 11444–11449.
[39] T. Taji, C. Ohsumi, S. Iuchi, M. Seki, M. Kasuga, M. Kobayashi, K. Yamaguchi-Shinozaki, K. Shinozaki, Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*, Plant J. 29 (2002) 417–426.
[40] J. Peng, D.E. Richards, N.M. Hartley, G.P. Murphy, K.M. Devos, J.E. Flintham, et al., "Green revolution" genes encode mutant gibberellin response modulators, Nature 400 (1999) 256–261.
[41] J. Lai, J. Messing, Increasing maize seed methionine by mRNA stability, Plant J. 30 (2002) 395–402.
[42] P. Lerouge, M. Bardor, S. Pagny, V. Gomord, L. Faye, N-Glycosylation of recombinant pharmaceutical glycoproteins produced in transgenic plants: towards an humanisation of plant *N*-glycans, Curr. Pharm. Biotechnol. 1 (2000) 347–354.
[43] D.J. Murphy, Biotechnological applications of seed biology, in: M. Black, K.J. Bradford, J. Vàsquez-Ramos (Eds.), Seed Biology: Advances and Applications, CAB International, 2000, pp. 427–438.
[44] D. Chargelegue, P. Obregon, P.M.W. Drake, Transgenic plants for vaccine production: expectations and limitations, Trends Plant Sci. 6 (2001) 495–496.
[45] L. Faye, N. Landry, P. Lerouge, V. Gomord, L.P. Vézina, La production de protéines à usage biopharmaceutique dans les plantes, Méd. Sci. 17 (2001) 867–877.
[46] S.J. Streatfield, J.A. Jilka, E.E. Hood, D.D. Turner, M.R. Bailey, J.M. Mayor, S.L. Woodar, K.K. Beifuss, M.E. Horn, D.E. Delaney, I.R. Tizard, J.A. Howard, Plant based vaccines: unique advantages, Vaccine 19 (2001) 2742–2748.
[47] K. Bohmert, I. Balbo, A. Steinbüchel, G. Tischendorf, L. Willmitzer, Constitutive expression of the β-ketothiolase gene in transgenic plants. A major obstacle for obtaining polyhydroxybutyrate-producing plants, Plant Physiol. 128 (2002) 1282–1290.
[48] T. Bouquin, M. Thomsen, L.K. Nielsen, T.H. Green, J. Mundy, M.H. Dziegie, Human anti-rhesus D IgG1 antibody produced in transgenic plants, Transgenic Res. 11 (2002) 115–122.
[49] L.E. Murray-Kolb, F. Takaiwa, F. Goto, T. Yoshihara, E.C. Theil, J.L. Beard, Transgenic rice is a source of iron for iron-depleted rats, J. Nutr. 132 (2002) 957–960.
[50] S.S. Sun, Methionine enhancement in plants, in: B.K. Sigh (Ed.), Plant Amino Acids. Biochemistry and Biotechnology, Marcel Dekker, 1999, pp. 509–521.
[51] X. Ye, S. Al-Babili, A. Kloti, J. Zang, P. Beyer, I. Potrykus, Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm, Science 287 (2000) 303–305.
[52] I. Potrykus, Golden rice and beyond, Plant Physiol. 125 (2001) 1157–1161.
[53] J. Machuka, Agricultural biotechnology for Africa. African scientists and farmers must feed their own people, Plant Physiol. 126 (2001) 16–19.