Review Article

Heterologous Expression of Plant Genes

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Heterologous expression allows the production of plant proteins in an organism which is simpler than the natural source. This technology is widely used for large-scale purification of plant proteins from microorganisms for biochemical and biophysical analyses. Additionally expression in well-defined model organisms provides insights into the functions of proteins in complex pathways. The present review gives an overview of recombinant plant protein production methods using bacteria, yeast, insect cells, and *Xenopus laevis* oocytes and discusses the advantages of each system for functional studies and protein characterization.

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1. Introduction

Heterologous expression involves identification of genes and transfer of the corresponding DNA fragments to hosts other than the original source for synthesis of the encoded proteins. Protein isolation, especially from plant sources, can be costly, cumbersome and lengthy, and heterologous expression provides a convenient alternative. This methodology allows large-scale production of plant proteins in microorganisms to study their biochemical and biophysical features. Foreign hosts may also provide a simpler system for studies on functions of proteins and for elucidation of their roles in complex mechanisms such as metabolic reactions and membrane transport. Recombinant plant proteins and peptides produced by heterologous expression are also used in industrial applications. Examples are provided by the synthesis of a medicinal peptide from ginseng as potential drug against diabetes [1] or production of plant lectins [2] in both cases in yeast.

The present review covers the recent literature on plant gene expression in bacteria, yeast, insect cells and *Xenopus* oocytes and presents the comparative advantages and disadvantages of each system. It also provides a survey of recent examples of application of heterologous expression technology to plant proteins. A comprehensive list of plant proteins expressed heterologously is given in Table 1. Factors influencing the choice of hosts, including the stability and folding characteristics of the protein, requirement for

posttranslational modifications, efficiency of the expression system, as well as simplicity and cost are discussed in the following sections.

2. Principal Components of Heterologous Expression

Basic principles of heterologous cloning and expression are summarized in Figure 1. Major parameters that affect choices at different stages are also indicated. The choice of the expression system and vector is a critical step in this procedure and, as indicated, advantages and disadvantages of several factors have to be considered. Expression systems are selected depending on whether the purpose of study is production of large quantities of protein or investigation of functional features of the cloned protein. The physicochemical properties of the investigated protein also play a role in this choice. A general review of frequently used expression systems is provided by Yin et al. [3].

A comprehensive survey of commercially available expression vectors has recently been published [4]. The most commonly used vectors are fusion systems that link additional amino acid sequences (tags) to the protein through a recognition site for a specific protease. Tags may consist of a short peptide sequence or a full protein which can be cleaved from the protein when desired. Presence of tag sequences facilitates solubility, purification, quantification, identification, localization, and assaying of the

FIGURE 1: Flow chart for heterologous expression.

expressed protein. Frequently used fusion partners include glutathione-S-transferase (GST), his-tag (poly-histidines), maltose binding protein (MBP), thioredoxin (TrxA), FLAG epitope-tag, c-Myc epitope-tag, disulfide isomerase I (DsbA), polyarginine-tag (Arg-tag), calmodulin-binding peptide, cellulose-binding domain, poly-histidine affinity tag (HAT-tag), N-utilizing substance-A (NusA), S-tag, streptavidin-binding peptide (SBP-tag), strep-tag, fluorescent proteins (e.g., green fluorescent protein (GFP)) and ubiquitin [4]. MBP and NusA are specifically used to increase the solubility. MBP is considered to be much more effective for enhancing solubility than GST and thioredoxin [5]. The major disadvantages of fusion protein systems are the requirement of expensive proteases for cleavage from the recombinant protein and the low yield of cleavage reactions [6].

Depending on the host system, vectors for transient or stable expression can be chosen as indicated below.

3. Expression Hosts

3.1. Prokaryotic Expression Systems

3.1.1. Escherichia Coli. *Escherichia coli* (*E. coli*) is the first and most extensively used prokaryotic expression system for heterologous protein production [7]. It remains generally the first choice due to its simplicity, rapid growth rate,

and relatively low cost. Almost all commercially available inducible cloning vectors are compatible with *E. coli* and extensive biochemical and genetic information is available.

One of the disadvantages of using *E. coli* as an expression host arises from its inability to perform post-translational modifications, which are often required for correct folding and functional activity of the recombinant protein. This applies particularly to some membrane proteins and enzymes [3]. Another disadvantage is that *E. coli* is generally not suitable for proteins which contain many disulfide bonds or require glycosylation, proline cis/trans isomerization, disulfide isomerization, lipidation, sulphation, or phosphorylation [8]. Some eukaryotic proteins that retain their full biological activity in the nonglycosylated form have, however, been produced in *E. coli*. The unglycosylated human growth hormone (hGH) binding protein secreted from *E. coli* retains the same binding affinity and specificity as the wild-type hGH binding protein suggesting that recombinant protein is properly folded and glycosylation is not required for binding [9].

Production of proteins that are stabilized by disulfide bonds in *E. coli* often results in proteolytic degradation or misfolding and formation of inclusion bodies [6]. One strategy developed to improve this situation is to target these proteins to the periplasm where the nonreducing environment allows formation of disulfide bonds [10, 11]. In

addition, the *E. coli* periplasm contains chaperone-like disulfide-binding proteins (DsbA, DsbB, DsbC, and DsbD), folding catalysts, and peptidyl-prolyl isomerases (SurA, RotA, FliB, and FkpA) that support disulfide bond formation and are important for correct folding of periplasmic proteins [12–14]. Disulfide bond formation is achieved via fusion to DsbA or DsbC [15, 16] and periplasmic secretion results in the functional production of a variety of recombinant proteins [17]. In a recent study, the rescue of unstable lipase B from *Pseudozyma antarctica* (PalB), with periplasmic folding factors was demonstrated [18]. Another strategy involves the use of the *trxB gor* double mutant lacking thioredoxin reductase and glutathione reductase genes [19, 20]. This double mutant was used for heterologous expression of barley oxalate oxidase (HvOXO) in *E. coli* [21]. The gene for an osmotin-like cryoprotective protein from *Solanum dulcamara* was expressed in *E. coli* and directed to periplasmic localization using an expression vector containing the *pelB* signal sequence [22]. This resulted in high concentrations of soluble protein with cryoprotective activity, whereas expression in the bacterial cytoplasm only yielded large amounts of insoluble and aggregated protein.

Some of the plant proteins accumulated in insoluble inclusion bodies in *E. coli* can be solubilized and refolded to restore activity after purification from the host. Examples include Arabidopsis thaumatin-like protein (ATLP3) which was purified from inclusion bodies and the refolded form displayed activity against some pathogenic fungi [23]. To validate the potential antifungal activity of *Solanum nigrum* osmotin-like protein (SnOLP) was overexpressed in *E. coli* and the recombinant protein was refolded using reduced/oxidized glutathione redox buffer and its *in vitro* activity was demonstrated [24]. The soybean RHG1-LRR domain protein was solubilized from inclusion bodies using urea and refolded by removing the urea in the presence of arginine and reduced/oxidized glutathione [25].

Many plant enzymes are expressed in insoluble inclusion bodies but it is still possible to obtain high yields of active forms for structural studies [26]. The mature polypeptide of FatB thioesterase from the developing seed tissues of *Madhuca butyracea* was characterized by heterologous expression in *E. coli* [27]. The functionality of the MbFatB in the heterologous system was revealed by the altered growth behavior and cell morphology of the bacteria due to the changes in the fatty acid profile. The maize chloroplast transglutaminase (TGZ) [26] and glutamylcysteine ligase (GCL) [28] were efficiently overexpressed in *E. coli*. Recently, DELLA proteins from both Arabidopsis and *Malus domestica*, which are involved in regulation of plant growth in response to phytohormonal signals, were isolated and expressed in *E. coli* [29].

Examples of functional expression of plant proteins in *E. coli* are provided mostly by studies on membrane proteins. A mutant with very low K^+ uptake was used as host for studies on the K^+ transporters AKT2 [30], AtKUP1-2 [31], AtHKT1 [32] from Arabidopsis and EcHKT1 and EcHKT2 from *Eucalyptus camaldulensis* [33]. In another example, *E. coli* C43 strain, which is suitable for expression of membrane proteins was used for functional characterization of chloroplast ATP/ADP transporter from Arabidopsis [34].

FIGURE 2: UV-visible absorption spectrum of GSTdMT at 2.7 mg/mL concentration in 20 mM HEPES buffer at pH 8.0. The charge transfer band between 240 and 260 nm due to Cd-S interaction is indicated by the arrow. The Cd/protein ratio is given in the inset.

The seagrass HAK K^+ transporters, CnHAK1 and CnHAK2 were also overexpressed in *E. coli* and it was found that CnHAK1, but not CnHAK2, mediated very rapid K^+ or Rb^+ influxes [35]. Using a dicarboxylate uptake-deficient *E. coli* mutant, a peptide transporter, AgDCAT1 from alder, was shown to be a dicarboxylate, including malate, succinate, fumarate, and oxaloacetate, transporter [36].

E. coli has also been used for expression of small plant proteins with a fusion partner. Metallothioneins (MTs), which are difficult to purify from natural sources because of their small molecular weight (7 kD), unusual amino acid sequences containing a large number of cysteines and their proteolytic susceptibility belong to this class. Several MTs including a Cd^{2+} binding Type 1 durum wheat metallothionein (dMT) [37], fava bean Type 1 and Type 2 MTs [38], Arabidopsis MT1, MT2 and MT3 proteins [39], Type 3 MT3-A from the oil palm [40], Type 2 MT, QsMT from *Quercus suber* [41] have been produced in *E. coli* mainly for structural analyses. Since the fusion constructs of durum MT with GST (GSTdMT) can be purified in well-defined oligomeric states they are used as model systems for studies on metal-binding and for structural analyses. Figure 2 illustrates that Cd-binding to GSTdMT can be detected by UV-visible spectroscopy. The metal content of GSTdMT was shown to be the same as that expected from dMT alone. An example of the shape models generated from X-ray solution scattering data for GSTdMT is shown in Figure 3, together with the fit to experimental data. The models support a fold for dMT similar to that expected for the free molecule [42, 43]. These results are in agreement with earlier work suggesting independent folding of GST and its fusion components [44] and indicate that recombinant fusion complexes are useful as model systems for structural studies.

3.2. Eukaryotic Expression Systems. Eukaryotic expression systems offer the possibility of posttranslational modifications and are often used for investigations of protein

TABLE 1: Heterologous expression of plant proteins grouped according to the host cells.

Protein expressed	Plant	Reference
Escherichia coli		
Lipase B (PalB)	<i>Pseudozyma antarctica</i>	[18]
Oxalate oxidase	<i>Hordeum vulgare</i> , <i>Triticum aestivum</i>	[21]
Osmotin-like cryoprotective protein	<i>Solanum dulcamara</i>	[22]
Thaumatococin-like protein (ATLP3)	<i>Arabidopsis thaliana</i>	[23]
Osmotin-like protein (SnOLP)	<i>Solanum nigrum</i>	[24]
RHG1-LRR domain	<i>Glycine max</i>	[25]
Chloroplast transglutaminase (TGZ)	<i>Zea mays</i>	[26]
FatB thioesterase	<i>Madhuca butyracea</i>	[27]
Glutamatecysteine ligase (GCL)	<i>Arabidopsis thaliana</i>	[28]
DELLA proteins	<i>Arabidopsis thaliana</i> , <i>Malus domestica</i>	[29]
K ⁺ transporters; KAT1, AKT2-3, AtKUP1/AtKT1/AtPOT1, AtKUP2/AtKT2/AtPOT2, AtHKT1	<i>Arabidopsis thaliana</i>	[30–32]
K ⁺ transporters, EcHKT1, EcHKT2	<i>Eucalyptus globulus</i>	[33]
ATP/ADP transporter	<i>Arabidopsis thaliana</i>	[34]
HAK K ⁺ transporters, CnHAK1, CnHAK2	<i>Cymodocea nodosa</i>	[35]
Peptide transporter family member, AgDCAT1	<i>Alnus glutinosa</i>	[36]
Type 1 MT, dMT	<i>Triticum durum</i>	[37]
Type 1 and Type 2 MTs	<i>Vicia faba</i>	[38]
MT1, MT2, and MT3	<i>Arabidopsis thaliana</i>	[39]
Type 3 MT3-A	<i>Elaeis guineensis</i>	[40]
Type 2 MT, QsMT	<i>Quercus suber</i>	[41]
Soybean seed ferritin	<i>Glycine max</i>	[126]
Saccharomyces cerevisiae		
H ⁺ -amino acid symporter and K ⁺ channel, KAT1	<i>Arabidopsis thaliana</i>	[47]
Phosphate transporters; AtPT1 and AtPT2	<i>Arabidopsis thaliana</i>	[48]
K ⁺ transporter, HvHAK1	<i>Hordeum vulgare</i>	[49]
K ⁺ transporters, AtKT1, and AtKT2, AtKUP1	<i>Arabidopsis thaliana</i>	[50, 51]
K ⁺ transporter, HKT1	<i>Triticum aestivum</i>	[52, 53]
Sulfate transporters, LeST1-1 and LeST1-2	<i>Lycopersicon esculentum</i>	[54]
Copper transporters, (COPT1–5)	<i>Arabidopsis thaliana</i>	[55]
Peptide transporter, AtPTR1	<i>Arabidopsis thaliana</i>	[56]
K ⁺ /H ⁺ antiporter, AtChx17	<i>Arabidopsis thaliana</i>	[57]

TABLE 1: Continued.

Protein expressed	Plant	Reference
Hexose transporters, VvHT4 and VvHT5	<i>Vitis vinifera</i>	[58]
Plasma membrane-localized H ⁺ /inositol symporter, AtINT2	<i>Arabidopsis thaliana</i>	[59]
High affinity GABA transporter, AtGAT1	<i>Arabidopsis thaliana</i>	[60]
Tonoplast Intrinsic Proteins, AtTIP2;1 and AtTIP2;3	<i>Arabidopsis thaliana</i>	[61]
Sorbitol transporters, PmPLT1 and PmPLT2	<i>Plantago major</i>	[62]
<i>Pichia pastoris</i>		
Nitrate reductase	<i>Spinacia oleracea</i> , <i>Zea mays</i>	[69]
Invertase	<i>Ipomoea batatas</i>	[70]
α 1,6-galactosyltransferase	<i>Trigonella foenum-graecum</i>	[71]
α 1,6-xylosyltransferase	<i>Arabidopsis thaliana</i>	[72]
Glycosyltransferases	<i>Arabidopsis thaliana</i> <i>Bos taurus</i> , <i>Drosophila melanogaster</i> , <i>Caenorhabditis elegans</i> , <i>Leucopersicon esculentum</i>	[73]
β -D-fructofuranosidase	<i>Oryza sativa</i>	[74]
Apyrase	<i>Solanum tuberosum</i>	[75]
Oxalate oxidases, HvOXO, TaOXO	<i>Hordeum vulgare</i> , <i>Triticum aestivum</i>	[76, 77]
Lectin	<i>Canavalia brasiliensis</i> , <i>Nicotiana tabacum</i>	[2, 79]
Low-affinity cation transporter (LCT1)	<i>Triticum aestivum</i>	[80]
2S albumin storage proteins (AL1 and AL3)	<i>Glycine max</i>	[127]
Baculovirus-mediated insect cell		
Patatin	<i>Solanum tuberosum</i>	[81]
Reductase isoforms, AR1 and AR2	<i>Arabidopsis thaliana</i>	[82]
Peroxisomal short-chain acyl-CoA oxidase A	<i>Arabidopsis thaliana</i>	[83]
Cyclin-dependent kinase A (CDKA)	<i>Arabidopsis thaliana</i>	[84]
NADH-cytochrome (Cyt) b5 reductase	<i>Arabidopsis thaliana</i>	[85]
Geranylgeranyltransferase-I (GGT-I)	<i>Arabidopsis thaliana</i>	[86]
Acyl-CoA synthetase	<i>Arabidopsis thaliana</i>	[87]
Homogentisate phytyltransferase	<i>Arabidopsis thaliana</i>	[88]
(+)-Abscisic Acid 8'-Hydroxylase	<i>Arabidopsis thaliana</i>	[89]
β 1,2-xylosyltransferase	<i>Arabidopsis thaliana</i>	[90]
Ethylene-inducing xylanase	<i>Nicotiana tabacum</i>	[91]

TABLE 1: Continued.

Protein expressed	Plant	Reference
ADP-glucose pyrophosphorylase (AGPase)	<i>Hordeum vulgare</i>	[92]
K ⁺ channels, AKT1, KAT1, and KCO1	<i>Arabidopsis thaliana</i>	[93–95]
K ⁺ channels KST1, SKT1, and KST1	<i>Solanum tuberosum</i>	[96, 97]
Transporter AUX1	<i>Arabidopsis thaliana</i>	[98]
β -phaseolin polypeptides	<i>Phaseolus vulgaris</i>	[128]
Ac-specific ORFa protein,	<i>Zea mays</i>	[129]
Cysteine protease papain	<i>Carica papaya</i>	[130]
Mitochondrial protein URF13	<i>Zea mays</i>	[131]
LAT52 protein	<i>Lycopersicon esculentum</i>	[132]
Auxin-binding protein (ABP1)	<i>Zea mays</i> , <i>Nicotiana tabacum</i>	[133, 134]
Calreticulin and auxin binding protein	<i>Zea mays</i>	[135]
Cinnamate 4-Hydroxylase	<i>Arabidopsis thaliana</i>	[136]
Cryptochrome-1	<i>Arabidopsis thaliana</i>	[137]
Phototropin 2	<i>Arabidopsis thaliana</i>	[138]
Histidinol dehydrogenase	<i>Brassica oleracea</i>	[139]
Putative soluble epoxide hydrolase (sEH)	<i>Solanum tuberosum</i>	[140]
Imidazoleglycerolphosphate dehydratase	<i>Arabidopsis thaliana</i>	[141]
Phytone synthase, Phytoene desaturase	<i>Narcissus pseudonarcissus</i>	[142, 143]
4-coumarate:coenzyme A ligase (4Cl)	<i>Populus trichocarpa</i> , <i>Populus deltoides</i>	[144]
Xenopus laevis oocytes		
Na ⁺ – K ⁺ cotransporter HKT1	<i>Arabidopsis thaliana</i>	[39]
AgDCAT1 nodule-specific transporter	<i>Alnus glutinosa</i>	[43]
AtNAR2.1/AtNRT2 Nitrate Transport System	<i>Arabidopsis thaliana</i>	[102]
HKT Constructs, AtHKT1_HKT1 chimeras	<i>Triticum aestivum</i> , <i>Arabidopsis thaliana</i>	[103]
HKT1 superfamily of K ⁺ /Na ⁺ transporters	<i>Eucalyptus camaldulensis</i>	[104]
Ammonium transporter, LeAMT1	<i>Lycopersicon esculentum</i>	[105]
Ammonium transporter, AtAMT1;2	<i>Arabidopsis thaliana</i>	[106]
Sucrose transporters, AtSUC2, AtSUC9, LjSUT4	<i>Arabidopsis thaliana</i> , <i>Lotus japonicus</i>	[107–109]
Al-activated malate transporter, BnALMT1, BnALMT2, ALMT1	<i>Brassica napus</i> , <i>Triticum aestivum</i>	[110, 111]
Polyol transporters, AtPLT5, PmPLT1	<i>Arabidopsis thaliana</i> , <i>Plantago major</i>	[112, 113]

TABLE 1: Continued.

Protein expressed	Plant	Reference
Inositol transporter2, AtINT2, AtINT4	<i>Arabidopsis thaliana</i>	[114, 115]
Amino acid transporter, AtCAT6,	<i>Arabidopsis thaliana</i>	[116]
Cation-Cl ⁻ cotransporter, CCC	<i>Arabidopsis thaliana</i>	[117]
Anion-selective transporter, ZmALMT1	<i>Zea mays</i>	[118]
K ⁺ channel, SIRC	<i>Vitis vinifera</i>	[145]
K ⁺ channel, KZM1	<i>Zea mays</i>	[146]
K ⁺ channel, ZMK1	<i>Zea mays</i>	[147]
K ⁺ channels, SKT1 and LKT1	<i>Solanum tuberosum</i> , <i>Lycopersicon esculentum</i>	[148]
AKT2-KAT2 subunitits	<i>Arabidopsis thaliana</i>	[149]
K ⁺ channel, KAT1	<i>Arabidopsis thaliana</i>	[150]
Cyclic nucleotide-gated ion channels AtCNGC2, AtCNGC1, -2	<i>Arabidopsis thaliana</i> , <i>Nicotiana tobacum</i>	[151, 152]
Putative transporter (GmN70)	<i>Glycine max</i>	[153]
Al-activated malate transporter, TaALMT1	<i>Triticum aestivum</i>	[154]
High affinity γ -aminobutyric acid transporter, AtGAT1	<i>Arabidopsis thaliana</i>	[155]
Aquaporins, ZmPIP1a, ZmPIP1b, ZmPIP2a, PIP1, ZmPIP2;1	<i>Zea mays</i>	[124, 156, 157]
Aquaporin, PIP1	<i>Lycopersicon esculentum</i>	[158]
Aquaporin, PIP2	<i>Juglans regia</i>	[159]
Tonoplast intrinsic protein, AtTIP2;1	<i>Arabidopsis thaliana</i>	[160, 161]
Aquaporin, McTIP1;2	<i>Mesembryanthemum crystallinum</i>	[162]
Aquaporin, HvPIP1;6	<i>Hordeum vulgare</i>	[163]
Tonoplast intrinsic protein, PgTIP1	<i>Panax ginseng</i>	[164]
Nodulin 26 intrinsic protein, AtNIP2;1	<i>Arabidopsis thaliana</i>	[165]
PIP-1-type; NtPIP1;1, NtAQP1; PIP-2-type; NtPIP2;1	<i>Nicotiana tabacum</i>	[166]
CjMDR1, ATP-binding cassette protein	<i>Coptis japonica</i>	[167]
GlpF-like intrinsic protein (GIP1;1),	<i>Physcomitrella patens</i>	[168]
Metal tolerance protein1, AtMTP1	<i>Arabidopsis thaliana</i>	[169]
AtTPK4 tandem-pore K ⁺ channel	<i>Arabidopsis thaliana</i>	[170]
FRD3, multidrug and toxin efflux (MATE)	<i>Arabidopsis thaliana</i>	[171]

FIGURE 3: A: a low resolution shape model for GSTdMT. The GST dimer (red and blue) is located at the center from which the dMT molecules extend (green). B: the scattering curve expected from the model (—) agrees well with the experimental data (...). $I(S)$ is the scattering intensity and S the scattering vector given by $S = 4\pi \sin \theta / \lambda$, where 2θ is the scattering angle and $\lambda = 1.5 \text{ \AA}$ is the wavelength of X-rays. The model and the expected scattering pattern were calculated using the programs in the ATSAS package (EMBL Hamburg Outstation).

function. Processing reactions such as *O*- and *N*-linked glycosylation, tyrosine, serine, and threonine phosphorylation, addition of fatty acid chains, processing of signal sequences, disulfide bond formation, and correct folding can all be readily performed in eukaryotic hosts. The most commonly used eukaryotic systems are yeast, insect, mammalian, and plant cells.

3.2.1. Yeast. As a single cell eukaryotic organism, yeast has molecular, genetic, and biochemical characteristics which are similar to those of higher eukaryotes, and is useful for heterologous protein production. Yeast cells can grow rapidly with high cell densities, and are easy to manipulate and yeast cultures are cost effective. The two most commonly used organisms are *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Pichia pastoris* (*P. pastoris*) [7].

Saccharomyces Cerevisiae. Baker's yeast, *S. cerevisiae*, is widely used as a host organism for heterologous expression of proteins. Its genetics and physiology are well documented and proteins are posttranslationally modified through the mechanisms similar to those found in plants. The limitations of this host system are low yields, cell stress due to the presence of the foreign gene and hyperglycosylation of secreted foreign proteins. Lack of a strong inducible promoter can be circumvented using *P. pastoris* [45].

Earlier work on heterologous expression for screening of plant cDNA libraries by complementation in *S. cerevisiae* null mutants was reviewed by Frommer and Ninnemann [7]. The *S. cerevisiae* mutants provide a convenient system for functional and kinetic studies of transporters [46]. The electrophysiological properties of membrane transporters, H^+ -amino acid symporter and K^+ channel, KAT1 [47] and phosphate transporters; AtPT1 and AtPT2 of Arabidopsis were characterized using *S. cerevisiae* [48]. Recently, functional expression of transporters such as an HvHAK1 from barley [49], AtKT1 and AtKT2 [50], and AtKUP1 from Ara-

bidopsis [51] also utilized *S. cerevisiae* mutants. Another K^+ transporter characterized in this system is HKT1 from wheat [52, 53]. Kinetic uptake analyses of tomato sulfate transporters, LeST1-1 and LeST1-2 were carried out using the *S. cerevisiae* sulfate transporter mutant [54]. The five members of the copper transporter family COPT1–5 from Arabidopsis were characterized using a copper transport null mutant [55]. A peptide transporter *AtPTR1* gene from Arabidopsis was isolated and complemented in a peptide transport-deficient mutant [56]. A putative K^+/H^+ antiporter, AtChx17 was heterologously expressed and characterized in an *S. cerevisiae kha1* deletion mutant [57]. To test their functional activity, the grapevine hexose transporters VvHT3, VvHT4, and VvHT5 were expressed in the *S. cerevisiae* mutant EBY.VW4000, which is deficient in glucose transport due to concurrent knock-out of 20 endogenous transporter genes [58]. Growth-based complementation assays were used to demonstrate function of the transporters but resulted in inadequate rates of glucose uptake. A more sensitive assay based on direct measurement of radioactively labelled glucose uptake revealed that this mutant expressing VvHT4 and VvHT5 accumulated labelled glucose at higher rates than yeast transformed with the empty vector, demonstrating the functionality of the glucose transporters. Although VvHT3:GFP (green fluorescent protein) fusion protein was targeted to the plasma membrane in plant cells, VvHT3 was found not to be functional in the yeast system [58]. Yeast expression studies were, in several instances, complemented by studies in other organisms to verify functional and kinetic properties of recombinant proteins. The plasma membrane-localized H^+ /inositol symporter AtINT2 of Arabidopsis was studied by expression in an inositol uptake/inositol biosynthesis double mutant in *S. cerevisiae* and in *Xenopus* oocytes [59]. In this study, the amount of AtINT2 protein in yeast plasma membrane was sufficient for complementation, but not for functional and kinetic analyses. In oocytes, however, it was possible to show that AtINT2 mediated the symport

of H^+ [59]. Expression and functional characterization of Arabidopsis AtGAT1 in *S. cerevisiae* and *Xenopus* oocytes revealed that AtGAT1 mediates H^+ -dependent, high affinity transport of high affinity γ -aminobutyric acid (GABA) and GABA-related compounds. Properties of this protein could be examined in more detail in *Xenopus* oocytes [60]. Heterologous expression of AtTIP2;1 and AtTIP2;3 from Arabidopsis in both ammonium uptake-defective yeast and oocytes indicated that these TIPs transport both ammonium and methyl-ammonium in addition to water and urea [61]. The kinetic characteristics of the sorbitol transporters, PmPLT1, and PmPLT2 from common plantain (*Plantago major*) were investigated by functional expression in *S. cerevisiae* and in *Xenopus* oocytes. In the yeast system, both proteins were characterized as low-affinity and low-specificity polyol symporters. These data were confirmed in the *Xenopus* system, where PmPLT1 was analyzed in detail and characterized as an H^+ symporter [62].

The major disadvantages of using *S. cerevisiae* mutants in transporter studies are the hyperpolarization of the membrane, mislocalization of membrane proteins and recruitment of non- K^+ -transporters into K^+ -transporters [63].

Pichia Pastoris. *P. pastoris*, methylotrophic yeast, is considered a valuable tool for high yield heterologous expression of various proteins. The possibility of obtaining posttranslational modifications, high level expression of foreign proteins in either intracellular or extracellular forms, simplicity of genetic manipulations, and availability of various *P. pastoris* strains and vectors make this expression system highly popular [64]. Molecular manipulations such as gene targeting, high frequency DNA transformation, and cloning for functional complementation are similar to those in *S. cerevisiae* [64]. Tightly regulated promoters, easy integration of heterologous DNA into the host chromosome and the capacity to generate more posttranslational modifications make *P. pastoris* the preferred system compared to *S. cerevisiae*.

The wide use of *P. pastoris* expression system for recombinant plant proteins can be seen from recent reviews [64, 65]. *P. pastoris* is particularly well suited for studying plant enzymes since glycosylation of the foreign proteins is expected to be closer to that in plants [66, 67] and glycosylated proteins have shorter glycosyl chains in *P. pastoris* than in *S. cerevisiae* [68]. This expression system has the potential to produce high levels of recombinant proteins [67], up to 400 mg/L of culture [69]. Several plant enzymes have been produced in *Pichia*. Two examples are cytosolic expression of nitrate reductase from spinach and corn at high levels needed for detailed biochemical studies [69] and expression of a sweet potato invertase in milligram quantities [70]. Enzymatic activity of the membrane-bound α 1,6-galactosyltransferase was shown through overexpression in *P. pastoris* [71]. The hypothesis that α -xylosyltransferase is involved in xyloglucan biosynthesis was tested by overexpressing the corresponding genes and identifying the gene product that displayed activity [72]. *P. pastoris* has been used for production of a number of glycosyltransferases involved in the biosynthesis of *N*- and *O*-linked oligosaccharides

[73]. To confirm that *Os β fruct3* from rice encoded a vacuolar type β -D-fructofuranosidase, the *Os β fruct3* cDNA was expressed in this host [74]. A recombinant potato apyrase was expressed and purified in the hyperglycosylated form at 1 mg/L protein concentration [75]. The catalytically active barley oxalate oxidase, HvOXO was produced with a yield of 50 mg/L culture and biochemically characterized [76].

High-level expression of wheat germin/oxalate oxidase was achieved in *P. pastoris* as an α -mating factor signal peptide fusion to increase secretion of the protein of interest into the culture medium. Approximately 1 g (4×10^4 U) of TaOXO was produced in 5 L fermentation cultures following 8 days of methanol induction, demonstrating the possibility of large-scale production of oxalate oxidase for biotechnological applications. Glycosylation of the recombinant protein was evidenced by mass spectrometry [77]. Another application using *P. pastoris* is the expression of the α -subunit of heterotrimeric G-proteins, GPA1, from Arabidopsis. Several attempts had previously failed to produce this protein in *E. coli*, whereas in the yeast system the protein could be expressed with a his_6 -tag and purified by affinity chromatography with a yield up to 20 mg from 700 mL culture [78].

Several allergens including, Cyn d 1 from Bermuda grass, Bla g 4 from German cockroach, Amb a 6 from *Ambrosia artemisiifolia*, and Ole e 1 from *Olea europaea* have also been produced in *P. pastoris* (see list in 64).

This system was also used for the expression of a number of plant lectins such as *Canavalia brasiliensis* lectin (ConBr) [2] and the *Nicotiana tabacum* lectin [79]. In a recent study, the low-affinity cation transporter (LCT1) from wheat was also expressed and functionally characterized using *P. pastoris* [80].

3.2.2. Insect Cells. Baculoviruses have been used for the synthesis of a wide variety of eukaryotic recombinant proteins in insect cells. In this expression system one of the nonessential viral genes is replaced with the target protein through homologous recombination. The resulting recombinant baculovirus is used to infect cultured insect cells and the heterologous genes can be expressed under the control of the extremely strong pPolh, polyhedron promoter in the late phase of infection.

The most common baculovirus used for expression studies is *Autographa californica* multiple capsid nucleopolyhedrovirus (AcMNPV) and the most frequently used host insects are *Spodoptera frugiperda* and *Trichoplusia ni*. This expression system produces high levels of recombinant proteins which are soluble, post-translationally modified, biologically active, and functional [81]. The virus is not pathogenic to vertebrates or plants. The main drawback of this system over the bacterial and yeast systems lies in the noncontinuous expression of the heterologous gene; every round of protein production needs reinfection [3].

This heterologous expression system is mainly used to investigate enzymatic mechanisms in plants. The most recent examples include the Arabidopsis reductase isoforms, AR1 and AR2 [82], peroxisomal short-chain acyl-CoA oxidase A [83], cyclin-dependent kinase A [84], NADH-cytochrome b5 reductase [85], geranylgeranyltransferase-I [86], acyl-CoA

synthetase [87], homogentisate phytyltransferase [88], (+)-abscisic acid 8'-hydroxylase [89], β 1,2-xylosyltransferase [90], tobacco ethylene-inducing xylanase [91], and barley ADP-glucose pyrophosphorylase [92]. The overall yield of heterologous proteins obtained with this system is usually lower than with *P. pastoris*.

Baculovirus-infected insect cells have been used as an alternative system to *Xenopus* oocytes for expression and characterization of plant channel proteins. Several channel proteins which were not functional in oocytes could be characterized in baculovirus-infected insect cells such as the K⁺ channel proteins AKT1 [93], KAT1 [94], KCO1 [95] from Arabidopsis, and KST1 [96] and SKT1 [97] from potato.

To investigate the interaction between AUX1 and its transport substrate indole-3-acetic acid (IAA) from Arabidopsis, an epitope-tagged version of AUX1 was expressed at high levels in a baculovirus expression system and suitable membrane fragments were prepared from baculovirus-infected insect cells for direct measurement of IAA binding to AUX1. AUX1-IAA interactions were determined using a radio-ligand binding assay to confirm that AUX1 was able to bind IAA with an affinity (K_d) of 2.6 mM, comparable with estimates of the K_m for IAA transport [98].

The main disadvantages of using baculovirus-infected insect cells are difficulties in constructing the expression vectors, requirements for more complex laboratory facilities and skills, and the short expression periods after infection.

3.2.3. *Xenopus Laevis* Oocytes. The oocytes of the South African clawed frog, *Xenopus laevis*, are also used for heterologous expression of eukaryotic genes. The mRNA for the target protein, introduced by microinjection into the cytoplasm, is translated and the protein is posttranslationally modified by the oocyte [99]. Direct injections of DNA into the nucleus are also possible, but the manipulations are difficult as the nucleus can easily be damaged in the process.

Investigations on membrane transport proteins can be readily performed on oocytes where techniques for electrophysiological measurements are well established. Although, a high proportion of cells express the foreign gene after injection variations in the quality of oocytes and in the ability of individual cells to produce the heterologous protein can cause problems. Oocytes are not suitable for preparing large quantities of proteins and the short expression period often leads to technical difficulties. The system can also not be sustained over long periods of time and is not suitable for stable expression [99, 100].

Xenopus oocytes have, however, provided a powerful heterologous expression system for animal as well as plant genes. The possibility of using *Xenopus* oocytes as heterologous expression systems for the identification of plant transporters was first demonstrated by the expression of the H⁺/glucose transporter STP1 from Arabidopsis [101]. It has, since, been mainly used for production of transporters including potassium channels, H⁺/hexose cotransporters, aquaporins, and chloride channels [99]. In addition, functional expression of a nitrate transporter [102], a K⁺/Na⁺ transporter [39, 103, 104], ammonium transporters [105, 106], sucrose transporters [107–109], Al-activated malate

transporters [110, 111], polyol transporters [112, 113], inositol transporters [114, 115], an amino acid transporter [116], a cation-Cl-cotransporter [117], and an anion-selective transporter [118] in *Xenopus* oocytes were investigated. Cases where channel proteins expressed in oocytes were not functional have also been reported. These include the K⁺ channels AKT1 from Arabidopsis [93, 119, 120], TaAKT1 from wheat [121], DKT1 from carrot [122], and OsAKT1 from rice [123]. The causes for the lack of function of these recombinant proteins are not clear.

Several studies have used expression of a wild type and its mutant forms in *Xenopus* oocytes to confirm the *in vivo* functions of plant proteins, especially transporters and plasma membrane intrinsic proteins (PIPs or aquaporins). To demonstrate whether or not the plant K⁺ channels form multimers, the wild type and a mutant were coexpressed in *Xenopus* oocytes [120]. Coexpression of tomato ammonium transporter (LeAMT1;1) and its mutant in *Xenopus* oocytes inhibited ammonium transport, suggesting homooligomerization [105]. In another study, the role of phosphorylation in the water channel activity of wild-type and mutant ZmPIP2;1 was studied in *Xenopus* oocytes [124].

In recent studies, the *Xenopus* oocyte expression system was used to investigate structure-function relationships. In one example, differences in the function of two cation transporters, wheat HKT1 and Arabidopsis AtHKT1, were investigated using a series of AtHKT1/HKT1 chimeras with point mutations [103].

4. Conclusions

Heterologous expression of plant genes in other host organisms has two main applications: (1) overexpression of the encoded protein, for biochemical and biophysical characterization and (2) expression of foreign genes for determination of the function of the encoded protein by complementing in a mutant host. Overexpression of recombinant proteins is usually carried out with a cleavable tag to simplify purification in large quantities. In contrast, complementation studies are carried out in null mutants to restore a missing activity *in vivo*.

Decisions on which expression vectors to use and the choice of the expression host depend on the particular application. In general *E. coli* is the first choice as host because of its simplicity, availability of expression vectors, cost effectiveness, and availability of extensive genetic information on this host. Alternative expression systems are used only if the recombinant protein is inactive due to lack of essential posttranslational modifications and when detailed studies on the recombinant protein function are planned. Yeast systems have the advantage of ease of manipulation and short generation time. *S. cerevisiae* has been extensively used for functional complementation, biochemical, and electrophysiological characterization of plant membrane and transporter proteins. *P. pastoris* is the preferred host for overexpression of several plant enzymes. Baculovirus-mediated insect cell expression offers the possibility for detailed investigations of plant enzymes and transporters. The oocyte from *Xenopus laevis* is often used for monitoring activity and biochemical

and electrophysiological characterization of plant plasma membrane transporter and pump proteins.

Heterologous expression is a powerful tool for functional and biochemical analyses of genes and gene families isolated from various organisms. It is particularly important for plants where the whole genome sequence is not available. This system will also provide *denovo* analysis. Its limitations, however, should be kept in mind, especially when interpreting the results in terms of the native structure and function of proteins. Major problems arise from misfolding and mislocalization of recombinant proteins in foreign hosts. Strategies developed to avoid misfolding of recombinant proteins include expression in periplasmic space, expression with a tag, and utilization of different hosts. Mislocalization, on the other hand, may occur because the recombinant protein may take over the function of the missing host protein [125]. Conclusions on function need to be tested in alternative hosts and eventually in the plant itself.

References

- [1] Y. Yan, J. Chen, and J. Li, "Overexpression of a small medicinal peptide from ginseng in the yeast *Pichia pastoris*," *Protein Expression and Purification*, vol. 29, no. 2, pp. 161–166, 2003.
- [2] W. M. Bezerra, C. P. Carvalho, R. A. Moreira, and T. B. Grangeiro, "Establishment of a heterologous system for the expression of *Canavalia brasiliensis* lectin: a model for the study of protein splicing," *Genetics and Molecular Research*, vol. 5, no. 1, pp. 216–223, 2006.
- [3] J. Yin, G. Li, X. Ren, and G. Herrler, "Select what you need: a comparative evaluation of the advantages and limitations of frequently used expression systems for foreign genes," *Journal of Biotechnology*, vol. 127, no. 3, pp. 335–347, 2007.
- [4] K. Terpe, "Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems," *Applied Microbiology and Biotechnology*, vol. 60, no. 5, pp. 523–533, 2003.
- [5] R. B. Kapust and D. S. Waugh, "*Escherichia coli* maltose-binding protein is uncommonly effective at promoting the solubility of polypeptides to which it is fused," *Protein Science*, vol. 8, no. 8, pp. 1668–1674, 1999.
- [6] F. Baneyx, "Recombinant protein expression in *Escherichia coli*," *Current Opinion in Biotechnology*, vol. 10, no. 5, pp. 411–421, 1999.
- [7] W. B. Frommer and O. Ninnemann, "Heterologous expression of genes in bacterial, fungal, animal, and plant cells," *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 46, pp. 419–444, 1995.
- [8] A. Lueking, C. Holz, C. Gotthold, H. Lehrach, and D. Cahill, "A system for dual protein expression in *Pichia pastoris* and *Escherichia coli*," *Protein Expression and Purification*, vol. 20, no. 3, pp. 372–378, 2000.
- [9] G. Fuh, M. G. Mulkerrin, S. Bass, et al., "The human growth hormone receptor. Secretion from *Escherichia coli* and disulfide bonding pattern of the extracellular binding domain," *The Journal of Biological Chemistry*, vol. 265, no. 6, pp. 3111–3115, 1990.
- [10] C. Wülfing and A. Plückthun, "Protein folding in the periplasm of *Escherichia coli*," *Molecular Microbiology*, vol. 12, no. 5, pp. 685–692, 1994.
- [11] G. Georgiou and L. Segatori, "Preparative expression of secreted proteins in bacteria: status report and future prospects," *Current Opinion in Biotechnology*, vol. 16, no. 5, pp. 538–545, 2005.
- [12] A. Shokri, A. M. Sandén, and G. Larsson, "Cell and process design for targeting of recombinant protein into the culture medium of *Escherichia coli*," *Applied Microbiology and Biotechnology*, vol. 60, no. 6, pp. 654–664, 2003.
- [13] F. Baneyx and M. Mujacic, "Recombinant protein folding and misfolding in *Escherichia coli*," *Nature Biotechnology*, vol. 22, no. 11, pp. 1399–1408, 2004.
- [14] J. H. Choi and S. Y. Lee, "Secretory and extracellular production of recombinant proteins using *Escherichia coli*," *Applied Microbiology and Biotechnology*, vol. 64, no. 5, pp. 625–635, 2004.
- [15] Y. Kurokawa, H. Yanagi, and T. Yura, "Overproduction of bacterial protein disulfide isomerase (DsbC) and its modulator (DsbD) markedly enhances periplasmic production of human nerve growth factor in *Escherichia coli*," *The Journal of Biological Chemistry*, vol. 276, no. 17, pp. 14393–14399, 2001.
- [16] S. Sahdev, S. K. Khattar, and K. S. Saini, "Production of active eukaryotic proteins through bacterial expression systems: a review of the existing biotechnology strategies," *Molecular and Cellular Biochemistry*, vol. 307, no. 1–2, pp. 249–264, 2008.
- [17] F. J. M. Mergulhão, D. K. Summers, and G. A. Monteiro, "Recombinant protein secretion in *Escherichia coli*," *Biotechnology Advances*, vol. 23, no. 3, pp. 177–202, 2005.
- [18] Y. Xu, D. Lewis, and C. P. Chou, "Effect of folding factors in rescuing unstable heterologous lipase B to enhance its overexpression in the periplasm of *Escherichia coli*," *Applied Microbiology and Biotechnology*, vol. 79, no. 6, pp. 1035–1044, 2008.
- [19] P. H. Bessette, F. Åslund, J. Beckwith, and G. Georgiou, "Efficient folding of proteins with multiple disulfide bonds in the *Escherichia coli* cytoplasm," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 24, pp. 13703–13708, 1999.
- [20] W. A. Prinz, F. Åslund, A. Holmgren, and J. Beckwith, "The role of the thioredoxin and glutaredoxin pathways in reducing protein disulfide bonds in the *Escherichia coli* cytoplasm," *The Journal of Biological Chemistry*, vol. 272, no. 25, pp. 15661–15667, 1997.
- [21] P. Cassland, S. Larsson, N.-O. Nilvebrant, and L. J. Jönsson, "Heterologous expression of barley and wheat oxalate oxidase in an *E. coli* *trx*B *gor* double mutant," *Journal of Biotechnology*, vol. 109, no. 1–2, pp. 53–62, 2004.
- [22] S. S. Newton and J. G. Duman, "An osmotin-like cryoprotective protein from the bittersweet nightshade *Solanum dulcamara*," *Plant Molecular Biology*, vol. 44, no. 5, pp. 581–589, 2000.
- [23] X. Hu and A. S. N. Reddy, "Cloning and expression of a PR5-like protein from *Arabidopsis*: inhibition of fungal growth by bacterially expressed protein," *Plant Molecular Biology*, vol. 34, no. 6, pp. 949–959, 1997.
- [24] M. de A Campos, M. S. Silva, C. P. Magalhães, et al., "Expression in *Escherichia coli*, purification, refolding and antifungal activity of an osmotin from *Solanum nigrum*," *Microbial Cell Factories*, vol. 7, pp. 7–17, 2008.
- [25] A. J. Afzal and D. A. Lightfoot, "Soybean disease resistance protein RHG1-LRR domain expressed, purified and refolded from *Escherichia coli* inclusion bodies: preparation for a functional analysis," *Protein Expression and Purification*, vol. 53, no. 2, pp. 346–355, 2007.
- [26] P. K. Carvajal-Vallejos, A. Campos, P. Fuentes-Prior, et al., "Purification and in vitro refolding of maize chloroplast

- transglutaminase over-expressed in *Escherichia coli*," *Biotechnology Letters*, vol. 29, no. 8, pp. 1255–1262, 2007.
- [27] J. K. Jha, M. K. Maiti, A. Bhattacharjee, A. Basu, P. C. Sen, and S. K. Sen, "Cloning and functional expression of an acyl-ACP thioesterase FatB type from *Diploknema (Madhuca) butyracea* seeds in *Escherichia coli*," *Plant Physiology and Biochemistry*, vol. 44, no. 11–12, pp. 645–655, 2006.
  - [28] J. M. Jez, R. E. Cahoon, and S. Chen, "Arabidopsis thaliana glutamate-cysteine ligase. Functional properties, kinetic mechanism, and regulation of activity," *The Journal of Biological Chemistry*, vol. 279, no. 32, pp. 33463–33470, 2004.
  - [29] X. Sun, N. Frearson, C. Kirk, et al., "An *E. coli* expression system optimized for DELLA proteins," *Protein Expression and Purification*, vol. 58, no. 1, pp. 168–174, 2008.
  - [30] N. Uozumi, T. Nakamura, J. I. Schroeder, and S. Muto, "Determination of transmembrane topology of an inward-rectifying potassium channel from *Arabidopsis thaliana* based on functional expression in *Escherichia coli*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 17, pp. 9773–9778, 1998.
  - [31] E. J. Kim, J. M. Kwak, N. Uozumi, and J. I. Schroeder, "AtKUP1: an *Arabidopsis* gene encoding high-affinity potassium transport activity," *The Plant Cell*, vol. 10, no. 1, pp. 51–62, 1998.
  - [32] N. Uozumi, E. J. Kim, F. Rubio, et al., "The *Arabidopsis* HKT1 gene homolog mediates inward Na⁺ currents in *Xenopus laevis* oocytes and Na⁺ uptake in *Saccharomyces cerevisiae*," *Plant Physiology*, vol. 122, no. 4, pp. 1249–1259, 2000.
  - [33] D. J. Fairbairn, W. Liu, D. P. Schachtman, S. Gomez-Gallego, S. R. Day, and R. D. Teasdale, "Characterisation of two distinct HKT1-like potassium transporters from *Eucalyptus camaldulensis*," *Plant Molecular Biology*, vol. 43, no. 4, pp. 515–525, 2000.
  - [34] J. Tjaden, C. Schwöppe, T. Möhlmann, P. W. Quick, and H. E. Neuhaus, "Expression of a plastidic ATP/ADP transporter gene in *Escherichia coli* leads to a functional adenine nucleotide transport system in the bacterial cytoplasmic membrane," *The Journal of Biological Chemistry*, vol. 273, no. 16, pp. 9630–9636, 1998.
  - [35] B. Garciadeblas, B. Benito, and A. Rodríguez-Navarro, "Molecular cloning and functional expression in bacteria of the potassium transporters CnHAK1 and CnHAK2 of the seagrass *Cymodocea nodosa*," *Plant Molecular Biology*, vol. 50, no. 4–5, pp. 623–633, 2002.
  - [36] J. Jeong, S. Suh, C. Guan, et al., "A nodule-specific dicarboxylate transporter from alder is a member of the peptide transporter family," *Plant Physiology*, vol. 134, no. 3, pp. 969–978, 2004.
  - [37] K. Bilecen, U. H. Ozturk, A. D. Duru, et al., "Triticum durum metallothionein: isolation of the gene and structural characterization of the protein using solution scattering and molecular modeling," *The Journal of Biological Chemistry*, vol. 280, no. 14, pp. 13701–13711, 2005.
  - [38] R. C. Foley, Z. M. Liang, and K. B. Singh, "Analysis of type 1 metallothionein cDNAs in *Vicia faba*," *Plant Molecular Biology*, vol. 33, no. 4, pp. 583–591, 1997.
  - [39] A. Murphy, J. Zhou, P. B. Goldsbrough, and L. Taiz, "Purification and immunological identification of metallothioneins 1 and 2 from *Arabidopsis thaliana*," *Plant Physiology*, vol. 113, no. 4, pp. 1293–1301, 1997.
  - [40] S. N. A. Abdullah, S. C. Cheah, and D. J. Murphy, "Isolation and characterisation of two divergent type 3 metallothioneins from oil palm, *Elaeis guineensis*," *Plant Physiology and Biochemistry*, vol. 40, no. 3, pp. 255–263, 2002.
  - [41] G. Mir, J. Domènech, G. Huguet, et al., "A plant type 2 metallothionein (MT) from cork tissue responds to oxidative stress," *Journal of Experimental Botany*, vol. 55, no. 408, pp. 2483–2493, 2004.
  - [42] F. Dede, G. Dinler, and Z. Sayers, "3D Macromolecular structure analyses: applications in plant proteins," in *Proceedings of the NATO Advanced Research Workshop*, pp. 135–146, Springer, 2006.
  - [43] F. Yesilirmak, *Biophysical and functional characterization of wheat metallothionein at molecular level*, Ph.D. thesis, Sabanci University, Istanbul, Turkey, 2008.
  - [44] Y. Zhan, X. Song, and G. W. Zhou, "Structural analysis of regulatory protein domains using GST-fusion proteins," *Gene*, vol. 281, no. 1–2, pp. 1–9, 2001.
  - [45] M. Schmidt and D. R. Hoffman, "Expression systems for production of recombinant allergens," *International Archives of Allergy and Immunology*, vol. 128, no. 4, pp. 264–270, 2002.
  - [46] I. Dreyer, C. Horeau, G. Lemaillé, et al., "Identification and characterization of plant transporters using heterologous expression systems," *Journal of Experimental Botany*, vol. 50, pp. 1073–1087, 1999.
  - [47] A. Bertl, J. A. Anderson, C. L. Slayman, and R. F. Gaber, "Use of *Saccharomyces cerevisiae* for patch-clamp analysis of heterologous membrane proteins: characterization of Kat1, an inward-rectifying K⁺ channel from *Arabidopsis thaliana*, and comparison with endogenous yeast channels and carriers," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 7, pp. 2701–2705, 1995.
  - [48] U. S. Muchhal, J. M. Pardo, and K. G. Raghothama, "Phosphate transporters from the higher plant *Arabidopsis thaliana*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 19, pp. 10519–10523, 1996.
  - [49] G. E. Santa-María, F. Rubio, J. Dubcovsky, and A. Rodríguez-Navarro, "The HAK1 gene of barley is a member of a large gene family and encodes a high-affinity potassium transporter," *The Plant Cell*, vol. 9, no. 12, pp. 2281–2289, 1997.
  - [50] F. J. Quintero and M. R. Blatt, "A new family of K⁺ transporters from *Arabidopsis* that are conserved across phyla," *FEBS Letters*, vol. 415, no. 2, pp. 206–211, 1997.
  - [51] H.-H. Fu and S. Luan, "AtKUP1: a dual-affinity K⁺ transporter from *Arabidopsis*," *The Plant Cell*, vol. 10, no. 1, pp. 63–73, 1998.
  - [52] D. P. Schachtman and J. I. Schroeder, "Structure and transport mechanism of a high-affinity potassium uptake transporter from higher plants," *Nature*, vol. 370, no. 6491, pp. 655–658, 1994.
  - [53] F. Rubio, W. Gassmann, and J. I. Schroeder, "Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance," *Science*, vol. 270, no. 5242, pp. 1660–1663, 1995.
  - [54] J. R. Howarth, P. Fourcroy, J.-C. Davidian, F. W. Smith, and M. J. Hawkesford, "Cloning of two contrasting high-affinity sulfate transporters from tomato induced by low sulfate and infection by the vascular pathogen *Verticillium dahliae*," *Planta*, vol. 218, no. 1, pp. 58–64, 2003.
  - [55] V. Sancenón, S. Puig, H. Mira, D. J. Thiele, and L. Peñarrubia, "Identification of a copper transporter family in *Arabidopsis thaliana*," *Plant Molecular Biology*, vol. 51, no. 4, pp. 577–587, 2003.
  - [56] D. Dietrich, U. Hammes, K. Thor, et al., "AtPTR1, a plasma membrane peptide transporter expressed during seed

- germination and in vascular tissue of *Arabidopsis*,” *The Plant Journal*, vol. 40, no. 4, pp. 488–499, 2004.
- [57] L. Maresova and H. Sychrova, “*Arabidopsis thaliana* CHX17 gene complements the *kha1* deletion phenotypes in *Saccharomyces cerevisiae*,” *Yeast*, vol. 23, no. 16, pp. 1167–1171, 2006.
- [58] M. A. Hayes, C. Davies, and I. B. Dry, “Isolation, functional characterization, and expression analysis of grapevine (*Vitis vinifera* L.) hexose transporters: differential roles in sink and source tissues,” *Journal of Experimental Botany*, vol. 58, no. 8, pp. 1985–1997, 2007.
- [59] S. Schneider, A. Schneidereit, P. Udvardi, et al., “*Arabidopsis* Inositol Transporter2 mediates H⁺ symport of different inositol epimers and derivatives across the plasma membrane,” *Plant Physiology*, vol. 145, no. 4, pp. 1395–1407, 2007.
- [60] A. Meyer, S. Eskandari, S. Grallath, and D. Rentsch, “AtGAT1, a high affinity transporter for γ -aminobutyric acid in *Arabidopsis thaliana*,” *The Journal of Biological Chemistry*, vol. 281, no. 11, pp. 7197–7204, 2006.
- [61] D. Loqué, U. Ludewig, L. Yuan, and N. von Wirén, “Tonoplast intrinsic proteins AtTIP2;1 and AtTIP2;3 facilitate NH₃ transport into the vacuole,” *Plant Physiology*, vol. 137, no. 2, pp. 671–680, 2005.
- [62] M. Ramsperger-Gleixner, D. Geiger, R. Hedrich, and N. Sauer, “Differential expression of sucrose transporter and polyol transporter genes during maturation of common plantain companion cells,” *Plant Physiology*, vol. 134, no. 1, pp. 147–160, 2004.
- [63] R. Madrid, M. J. Gómez, J. Ramos, and A. Rodríguez-Navarro, “Ectopic potassium uptake in *trk1 trk2* mutants of *Saccharomyces cerevisiae* correlates with a highly hyperpolarized membrane potential,” *The Journal of Biological Chemistry*, vol. 273, no. 24, pp. 14838–14844, 1998.
- [64] J. L. Cereghino and J. M. Cregg, “Heterologous protein expression in the methylotrophic yeast *Pichia pastoris*,” *FEMS Microbiology Reviews*, vol. 24, no. 1, pp. 45–66, 2000.
- [65] S. Macauley-Patrick, M. L. Fazenda, B. McNeil, and L. M. Harvey, “Heterologous protein production using the *Pichia pastoris* expression system,” *Yeast*, vol. 22, no. 4, pp. 249–270, 2005.
- [66] L. S. Grinna and J. F. Tschopp, “Size distribution and general structural features of N-linked oligosaccharides from the methylotrophic yeast, *Pichia pastoris*,” *Yeast*, vol. 5, no. 2, pp. 107–115, 1989.
- [67] J. M. Cregg, T. S. Vedvick, and W. C. Raschke, “Recent advances in the expression of foreign genes in *Pichia pastoris*,” *Nature Biotechnology*, vol. 11, no. 8, pp. 905–910, 1993.
- [68] R. K. Bretthauer and F. J. Castellino, “Glycosylation of *Pichia pastoris*-derived proteins,” *Biotechnology and Applied Biochemistry*, vol. 30, no. 3, pp. 193–200, 1999.
- [69] J. A. Mertens, N. Shiraishi, and W. H. Campbell, “Recombinant expression of molybdenum reductase fragments of plant nitrate reductase at high levels in *Pichia pastoris*,” *Plant Physiology*, vol. 123, no. 2, pp. 743–756, 2000.
- [70] W.-C. Huang, A.-Y. Wang, L.-T. Wang, and H.-Y. Sung, “Expression and characterization of sweet potato invertase in *Pichia pastoris*,” *Journal of Agricultural and Food Chemistry*, vol. 51, no. 5, pp. 1494–1499, 2003.
- [71] M. E. Edwards, C. A. Dickson, S. Chengappa, C. Sidebottom, M. J. Gidley, and J. S. G. Reid, “Molecular characterisation of a membrane-bound galactosyltransferase of plant cell wall matrix polysaccharide biosynthesis,” *The Plant Journal*, vol. 19, no. 6, pp. 691–697, 1999.
- [72] A. Faik, N. J. Price, N. V. Raikhel, and K. Keegstra, “An *Arabidopsis* gene encoding an α -xylosyltransferase involved in xyloglucan biosynthesis,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 11, pp. 7797–7802, 2002.
- [73] M. Bencúrová, D. Rendić, G. Fabini, E.-M. Kopecky, F. Altmann, and I. B. H. Wilson, “Expression of eukaryotic glycosyltransferases in the yeast *Pichia pastoris*,” *Biochimie*, vol. 85, no. 3–4, pp. 413–422, 2003.
- [74] R.-H. Fu, A.-Y. Wang, Y.-C. Wang, and H.-Y. Sung, “A cDNA encoding vacuolar type β -D-fructofuranosidase (*Osβfruct3*) of rice and its expression in *Pichia pastoris*,” *Biotechnology Letters*, vol. 25, no. 18, pp. 1525–1530, 2003.
- [75] N. Nourizad, M. Ehn, B. Gharizadeh, S. Hober, and P. Nyrén, “Methylotrophic yeast *Pichia pastoris* as a host for production of ATP-diphosphohydrolase (apyrase) from potato tubers (*Solanum tuberosum*),” *Protein Expression and Purification*, vol. 27, no. 2, pp. 229–237, 2003.
- [76] M. M. Whittaker and J. W. Whittaker, “Characterization of recombinant barley oxalate oxidase expressed by *Pichia pastoris*,” *Journal of Biological Inorganic Chemistry*, vol. 7, no. 1–2, pp. 136–145, 2002.
- [77] H.-Y. Pan, M. M. Whittaker, R. Bouveret, A. Berna, F. Bernier, and J. W. Whittaker, “Characterization of wheat germin (oxalate oxidase) expressed by *Pichia pastoris*,” *Biochemical and Biophysical Research Communications*, vol. 356, no. 4, pp. 925–929, 2007.
- [78] B. Kaplan, S. Tunca, and Z. Sayers, “Expression of A. *thaliana* G protein alpha subunit in *P. pastoris*,” *FEBS Journal*, vol. 272, supplement 1, pp. 1–11, 2005.
- [79] N. Lannoo, W. Vervecken, P. Proost, P. Rougé, and E. J. M. Van Damme, “Expression of the nucleocytoplasmic tobacco lectin in the yeast *Pichia pastoris*,” *Protein Expression and Purification*, vol. 53, no. 2, pp. 275–282, 2007.
- [80] E. Diatloff, B. G. Forde, and S. K. Roberts, “Expression and transport characterisation of the wheat low-affinity cation transporter (LCT1) in the methylotrophic yeast *Pichia pastoris*,” *Biochemical and Biophysical Research Communications*, vol. 344, no. 3, pp. 807–813, 2006.
- [81] D. L. Andrews, B. Beames, M. D. Summers, and W. D. Park, “Characterization of the lipid acyl hydrolase activity of the major potato (*Solanum tuberosum*) tuber protein, patatin, by cloning and abundant expression in a baculovirus vector,” *Biochemical Journal*, vol. 252, no. 1, pp. 199–206, 1988.
- [82] M. Mizutani and D. Ohta, “Two isoforms of NADPH: cytochrome p450 reductase in *Arabidopsis thaliana* gene structure, heterologous expression in insect cells, and differential regulation,” *Plant Physiology*, vol. 116, no. 1, pp. 357–367, 1998.
- [83] H. Hayashi, L. De Bellis, A. Ciurli, M. Kondo, M. Hayashi, and M. Nishimura, “A novel Acyl-CoA oxidase that can oxidize short-chain Acyl-CoA in plant peroxisomes,” *The Journal of Biological Chemistry*, vol. 274, no. 18, pp. 12715–12721, 1999.
- [84] H. Harashima, A. Shinmyo, and M. Sekine, “Phosphorylation of threonine 161 in plant cyclin-dependent kinase A is required for cell division by activation of its associated kinase,” *The Plant Journal*, vol. 52, no. 3, pp. 435–448, 2007.
- [85] M. Fukuchi-Mizutani, M. Mizutani, Y. Tanaka, T. Kusumi, and D. Ohta, “Microsomal electron transfer in higher plants: cloning and heterologous expression of NADH-cytochrome

- b₅ reductase from *Arabidopsis*," *Plant Physiology*, vol. 119, no. 1, pp. 353–361, 1999.
- [86] D. Caldelari, H. Sternberg, M. Rodríguez-Concepción, W. Gruissem, and S. Yalovsky, "Efficient prenylation by a plant geranylgeranyltransferase-I requires a functional Caal box motif and a proximal polybasic domain," *Plant Physiology*, vol. 126, no. 4, pp. 1416–1429, 2001.
- [87] H. Hayashi, L. De Bellis, Y. Hayashi, et al., "Molecular characterization of an *Arabidopsis* acyl-coenzyme A synthetase localized on glyoxysomal membranes," *Plant Physiology*, vol. 130, no. 4, pp. 2019–2026, 2002.
- [88] B. Savidge, J. D. Weiss, Y.-H. H. Wong, et al., "Isolation and characterization of homogentisate phytyltransferase genes from *Synechocystis* sp. PCC 6803 and *Arabidopsis*," *Plant Physiology*, vol. 129, no. 1, pp. 321–332, 2002.
- [89] S. Saito, N. Hirai, C. Matsumoto, et al., "Arabidopsis CYP707As encode (+)-abscisic acid 8'-hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid," *Plant Physiology*, vol. 134, no. 4, pp. 1439–1449, 2004.
- [90] S. Pagny, F. Bouissonnie, M. Sarkar, et al., "Structural requirements for Arabidopsis β 1, 2-xylosyltransferase activity and targeting to the Golgi," *The Plant Journal*, vol. 33, no. 1, pp. 189–203, 2003.
- [91] N. Furman-Matarasso, E. Cohen, Q. Du, N. Chejanovsky, U. Hanania, and A. Avni, "A point mutation in the ethylene-inducing xylanase elicitor inhibits the β -1-4-endoxylanase activity but not the elicitation activity," *Plant Physiology*, vol. 121, no. 2, pp. 345–351, 1999.
- [92] D. N. P. Doan, H. Rudi, and O.-A. Olsen, "The allosterically unregulated isoform of ADP-glucose pyrophosphorylase from barley endosperm is the most likely source of ADP-glucose incorporated into endosperm starch," *Plant Physiology*, vol. 121, no. 3, pp. 965–975, 1999.
- [93] F. Gaymard, M. Cerutti, C. Horeau, et al., "The baculovirus/insect cell system as an alternative to *Xenopus* oocytes. First characterization of the AKT1 K⁺ channel from *Arabidopsis thaliana*," *The Journal of Biological Chemistry*, vol. 271, no. 37, pp. 22863–22870, 1996.
- [94] I. Marten, F. Gaymard, G. Lemaillet, J.-B. Thibaud, H. Sentenac, and R. Hedrich, "Functional expression of the plant K⁺ channel KAT1 in insect cells," *FEBS Letters*, vol. 380, no. 3, pp. 229–232, 1996.
- [95] K. Czempinski, S. Zimmermann, T. Ehrhardt, and B. Müller-Röber, "New structure and function in plant K⁺ channels: KCO1, an outward rectifier with a steep Ca²⁺ dependency," *The EMBO Journal*, vol. 16, no. 10, pp. 2565–2575, 1997.
- [96] T. Ehrhardt, S. Zimmermann, and B. Müller-Röber, "Association of plant K⁺ (in) channels is mediated by conserved and does not affect subunit assembly," *FEBS Letters*, vol. 409, no. 2, pp. 166–170, 1997.
- [97] S. Zimmermann, I. Talke, T. Ehrhardt, G. Nast, and B. Müller-Röber, "Characterization of SKT1, an inwardly rectifying potassium channel from potato, by heterologous in insect cells," *Plant Physiology*, vol. 116, no. 3, pp. 879–890, 1998.
- [98] D. J. Carrier, N. T. A. Bakar, R. Swarup, et al., "The binding of auxin to the *Arabidopsis* auxin influx transporter AUX1," *Plant Physiology*, vol. 148, no. 1, pp. 529–535, 2008.
- [99] A. J. Miller and J. J. Zhou, "Xenopus oocytes as an expression system for plant transporters," *Biochimica et Biophysica Acta*, vol. 1465, no. 1–2, pp. 343–358, 2000.
- [100] E. Sigel, "The *Xenopus* oocyte: system for the study of functional expression and modulation of proteins," *Molecular Nutrition and Food Research*, vol. 49, no. 3, pp. 228–234, 2005.
- [101] K. J. Boorer, B. G. Forde, R. A. Leigh, and A. J. Miller, "Functional expression of a plant plasma membrane transporter in *Xenopus* oocytes," *FEBS Letters*, vol. 302, no. 2, pp. 166–168, 1992.
- [102] M. Orsel, F. Chopin, O. Leleu, et al., "Characterization of a two-component high-affinity nitrate uptake system in *Arabidopsis*. Physiology and protein-protein interaction," *Plant Physiology*, vol. 142, no. 3, pp. 1304–1317, 2006.
- [103] W. Liu, D. J. Fairbairn, R. J. Reid, and D. P. Schachtman, "Characterization of two HKT1 homologues from *Eucalyptus camaldulensis* that display intrinsic osmosensing capability," *Plant Physiology*, vol. 127, no. 1, pp. 283–294, 2001.
- [104] P. Mäser, Y. Hosoo, S. Goshima, et al., "Glycine residues in potassium channel-like selectivity filters determine potassium selectivity in four-loop-per-subunit HKT transporters from plants," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 9, pp. 6428–6433, 2002.
- [105] U. Ludewig, S. Wilken, B. Wu, et al., "Homo- and heterooligomerization of ammonium transporter-1 NH₄⁺ uniporters," *The Journal of Biological Chemistry*, vol. 278, no. 46, pp. 45603–45610, 2003.
- [106] B. Neuhäuser, M. Dynowski, M. Mayer, and U. Ludewig, "Regulation of NH₄⁺ transport by essential cross talk between AMT monomers through the carboxyl tails," *Plant Physiology*, vol. 143, no. 4, pp. 1651–1659, 2007.
- [107] D. Chandran, A. Reinders, and J. M. Ward, "Substrate specificity of the *Arabidopsis thaliana* sucrose transporter AtSUC2," *The Journal of Biological Chemistry*, vol. 278, no. 45, pp. 44320–44325, 2003.
- [108] A. B. Sivitz, A. Reinders, M. E. Johnson, et al., "Arabidopsis sucrose transporter AtSUC9. High-affinity transport activity, intragenic control of expression, and early flowering mutant phenotype," *Plant Physiology*, vol. 143, no. 1, pp. 188–198, 2007.
- [109] A. Reinders, A. B. Sivitz, C. G. Starker, J. S. Gantt, and J. M. Ward, "Functional analysis of LjSUT4, a vacuolar sucrose transporter from *Lotus japonicus*," *Plant Molecular Biology*, vol. 68, no. 3, pp. 289–299, 2008.
- [110] T. Sasaki, Y. Yamamoto, B. Ezaki, et al., "A wheat gene encoding an aluminum-activated malate transporter," *The Plant Journal*, vol. 37, no. 5, pp. 645–653, 2004.
- [111] A. Ligaba, M. Katsuhara, P. R. Ryan, M. Shibusaka, and H. Matsumoto, "The BnALMT1 and BnALMT2 genes from rape encode aluminum-activated malate transporters that enhance the aluminum resistance of plant cells," *Plant Physiology*, vol. 142, no. 3, pp. 1294–1303, 2006.
- [112] Y.-S. Klepek, D. Geiger, R. Stadler, et al., "Arabidopsis POLYOL TRANSPORTERS, a new member of the monosaccharide transporter-like superfamily, mediates H⁺-symport of numerous substrates, including myo-inositol, glycerol, and ribose," *The Plant Cell*, vol. 17, no. 1, pp. 204–218, 2005.
- [113] M. Ramsperger-Gleixner, D. Geiger, R. Hedrich, and N. Sauer, "Differential expression of sucrose transporter and polyol transporter genes during maturation of common plantain companion cells," *Plant Physiology*, vol. 134, no. 1, pp. 147–160, 2004.
- [114] S. Schneider, A. Schneidereit, K. R. Konrad, et al., "Arabidopsis INOSITOL TRANSPORTER4 mediates high-affinity H⁺ symport of myoinositol across the plasma membrane," *Plant Physiology*, vol. 141, no. 2, pp. 567–577, 2006.

- [115] S. Schneider, A. Schneidereit, P. Udvardi, et al., "Arabidopsis INOSITOL TRANSPORTER2 mediates H⁺ symport of different inositol epimers and derivatives across the plasma membrane," *Plant Physiology*, vol. 145, no. 4, pp. 1395–1407, 2007.
- [116] U. Z. Hammes, E. Nielsen, L. A. Honaas, C. G. Taylor, and D. P. Schachtman, "AtCAT6, a sink-tissue-localized transporter for essential amino acids in *Arabidopsis*," *The Plant Journal*, vol. 48, no. 3, pp. 414–426, 2006.
- [117] J. M. Colmenero-Flores, G. Martínez, G. Gamba, et al., "Identification and functional characterization of cation-chloride cotransporters in plants," *The Plant Journal*, vol. 50, no. 2, pp. 278–292, 2007.
- [118] M. A. Piñeros, G. M. A. Cançado, L. G. Maron, S. M. Lyi, M. Menossi, and L. V. Kochian, "Not all ALMT1-type transporters mediate aluminum-activated organic acid responses: the case of *ZmALMT1*—an anion-selective transporter," *The Plant Journal*, vol. 53, no. 2, pp. 352–367, 2008.
- [119] H. Sentenac, N. Bonneaud, M. Minet, et al., "Cloning and expression in yeast of a plant potassium ion transport system," *Science*, vol. 256, no. 5057, pp. 663–665, 1992.
- [120] I. Dreyer, S. Antunes, T. Hoshi, et al., "Plant K⁺ channel α -subunits assemble indiscriminately," *Biophysical Journal*, vol. 72, no. 5, pp. 2143–2150, 1997.
- [121] P. H. Buschmann, R. Vaidyanathan, W. Gassmann, and J. I. Schroeder, "Enhancement of Na⁺ uptake currents, time-dependent inward-rectifying K⁺ channel currents, and K⁺ channel transcripts by K⁺ starvation in wheat root cells," *Plant Physiology*, vol. 122, no. 4, pp. 1387–1397, 2000.
- [122] E. Formentin, S. Varotto, A. Costa, et al., "DKT1, a novel K⁺ channel from carrot, forms functional heteromeric channels with KDC1," *FEBS Letters*, vol. 573, no. 1–3, pp. 61–67, 2004.
- [123] I. Fuchs, S. Stölzle, N. Ivashikina, and R. Hedrich, "Rice K⁺ uptake channel OsAKT1 is sensitive to salt stress," *Planta*, vol. 221, no. 2, pp. 212–221, 2005.
- [124] V. Van Wilder, U. Micielica, H. Degand, R. Derua, E. Waelkens, and F. Chaumont, "Maize plasma membrane aquaporins belonging to the PIP1 and PIP2 subgroups are in vivo phosphorylated," *Plant and Cell Physiology*, vol. 49, no. 9, pp. 1364–1377, 2008.
- [125] D. C. Bassham and N. V. Raikhel, "Plant cells are not just green yeast," *Plant Physiology*, vol. 122, no. 4, pp. 999–1001, 2000.
- [126] X. Dong, B. Tang, J. Li, Q. Xu, S. Fang, and Z. Hua, "Expression and purification of intact and functional soybean (*Glycine max*) seed ferritin complex in *Escherichia coli*," *Journal of Microbiology and Biotechnology*, vol. 18, no. 2, pp. 299–307, 2008.
- [127] J. Lin, R. Fido, P. Shewry, D. B. Archer, and M. J. C. Alcocer, "The expression and processing of two recombinant 2S albumins from soybean (*Glycine max*) in the yeast *Pichia pastoris*," *Biochimica et Biophysica Acta*, vol. 1698, no. 2, pp. 203–212, 2004.
- [128] M. M. Bustos, V. A. Luckow, L. R. Griffing, M. D. Summers, and T. C. Hall, "Expression, glycosylation and secretion of phaseolin in a baculovirus system," *Plant Molecular Biology*, vol. 10, no. 6, pp. 475–488, 1988.
- [129] R. Kunze and P. Starlinger, "The putative transposase of transposable element Ac from *Zea mays* L. interacts with subterminal sequences of Ac," *The EMBO Journal*, vol. 8, no. 11, pp. 3177–3185, 1989.
- [130] T. Vernet, D. C. Tessier, C. Richardson, et al., "Secretion of functional papain precursor from insect cells. Requirement for N-glycosylation of the pro-region," *The Journal of Biological Chemistry*, vol. 265, no. 27, pp. 16661–16666, 1990.
- [131] K. L. Korth and C. S. Levings III, "Baculovirus expression of the maize mitochondrial protein URF13 confers insecticidal activity in cell cultures and larvae," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 8, pp. 3388–3392, 1993.
- [132] J. Muschietti, L. Dircks, G. Vancanneyt, and S. McCormick, "LAT52 protein is essential for tomato pollen development: pollen expressing antisense *LAT52* RNA hydrates and germinates abnormally and cannot achieve fertilization," *The Plant Journal*, vol. 6, no. 3, pp. 321–328, 1994.
- [133] H. MacDonald, J. Henderson, R. M. Napier, M. A. Venis, C. Hawes, and C. M. Lazarus, "Authentic processing and targeting of active maize auxin-binding protein in the baculovirus expression system," *Plant Physiology*, vol. 105, no. 4, pp. 1049–1057, 1994.
- [134] J. M. Baully, I. M. Sealy, H. Macdonald, et al., "Overexpression of auxin-binding protein enhances the sensitivity of guard cells to auxin," *Plant Physiology*, vol. 124, no. 3, pp. 1229–1238, 2000.
- [135] H. Y. Meller Harel, V. Fontaine, H. Chen, I. M. Jones, and P. A. Millner, "Display of a maize cDNA library on baculovirus infected insect cells," *BMC Biotechnology*, vol. 8, pp. 64–69, 2008.
- [136] M. Mizutani, D. Ohta, and R. Sato, "Isolation of a cDNA and a genomic clone encoding cinnamate 4-hydroxylase from *Arabidopsis* and its expression manner in planta," *Plant Physiology*, vol. 113, no. 3, pp. 755–763, 1997.
- [137] J.-P. Bouly, B. Giovani, A. Djamei, et al., "Novel ATP-binding and autophosphorylation activity associated with *Arabidopsis* and human cryptochrome-1," *European Journal of Biochemistry*, vol. 270, no. 14, pp. 2921–2928, 2003.
- [138] H.-Y. Cho, T.-S. Tseng, E. Kaiserli, S. Sullivan, J. M. Christie, and W. R. Briggs, "Physiological roles of the light, oxygen, or voltage domains of phototropin 1 and phototropin 2 in *Arabidopsis*," *Plant Physiology*, vol. 143, no. 1, pp. 517–529, 2007.
- [139] A. Nagai, K. Suzuki, E. Ward, et al., "Overexpression of plant histidinol dehydrogenase using a baculovirus expression vector system," *Archives of Biochemistry and Biophysics*, vol. 295, no. 2, pp. 235–239, 1992.
- [140] A. Stapleton, J. K. Beetham, F. Pinot, et al., "Cloning and expression of soluble epoxide hydrolase from potato," *The Plant Journal*, vol. 6, no. 2, pp. 251–258, 1994.
- [141] S. Tada, M. Hatano, Y. Nakayama, et al., "Insect cell expression of recombinant imidazoleglycerolphosphate dehydratase of *Arabidopsis* and wheat and inhibition by triazole herbicides," *Plant Physiology*, vol. 109, no. 1, pp. 153–159, 1995.
- [142] M. Schledz, S. Al-Babili, J. von Lintig, et al., "Phytoene synthase from *Narcissus pseudonarcissus*: functional expression, galactolipid requirement, topological distribution in chromoplasts and induction during flowering," *The Plant Journal*, vol. 10, no. 5, pp. 781–792, 1996.
- [143] S. Al-Babili, J. von Lintig, H. Haubruck, and P. Beyer, "A novel, soluble form of phytoene desaturase from *Narcissus pseudonarcissus* chromoplasts is Hsp70-complexed and competent for flavinylation, membrane association and enzymatic activation," *The Plant Journal*, vol. 9, no. 5, pp. 601–612, 1996.
- [144] S. M. Allina, A. Pri-Hadash, D. A. Theilmann, B. E. Ellis, and C. J. Douglas, "4-coumarate:coenzyme a ligase in hybrid poplar. Properties of native enzymes, cDNA cloning, and

- analysis of recombinant enzymes," *Plant Physiology*, vol. 116, no. 2, pp. 743–754, 1998.
- [145] R. Pratelli, B. Lacombe, L. Torregrosa, et al., "A grapevine gene encoding a guard cell K^+ channel displays developmental regulation in the grapevine berry," *Plant Physiology*, vol. 128, no. 2, pp. 564–577, 2002.
- [146] K. Philippar, K. Büchsenschütz, M. Abshagen, et al., "The K^+ channel KZM1 mediates potassium uptake into the phloem and guard cells of the C_4 grass *Zea mays*," *The Journal of Biological Chemistry*, vol. 278, no. 19, pp. 16973–16981, 2003.
- [147] K. Philippar, I. Fuchs, H. Lüthen, et al., "Auxin-induced K^+ channel expression represents an essential step in coleoptile growth and gravitropism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 21, pp. 12186–12191, 1999.
- [148] S. Hartje, S. Zimmermann, D. Klonus, and B. Mueller-Roeber, "Functional characterisation of LKT1, a K^+ uptake channel from tomato root hairs, and comparison with the closely related potato inwardly rectifying K^+ channel SKT1 after expression in *Xenopus* oocytes," *Planta*, vol. 210, no. 5, pp. 723–731, 2000.
- [149] J. Xicluna, B. Lacombe, I. Dreyer, et al., "Increased functional diversity of plant K^+ channels by preferential heteromerization of the Shaker-like subunits AKT2 and KAT2," *The Journal of Biological Chemistry*, vol. 282, no. 1, pp. 486–494, 2007.
- [150] B. Sottocornola, S. Visconti, S. Orsi, et al., "The potassium channel KAT1 is activated by plant and animal 14-3-3 proteins," *The Journal of Biological Chemistry*, vol. 281, no. 47, pp. 35735–35741, 2006.
- [151] Q. Leng, R. W. Mercier, B.-G. Hua, H. Fromm, and G. A. Berkowitz, "Electrophysiological analysis of cloned cyclic nucleotide-gated ion channels," *Plant Physiology*, vol. 128, no. 2, pp. 400–410, 2002.
- [152] B.-G. Hua, R. W. Mercier, Q. Leng, and G. A. Berkowitz, "Plants do it differently. A new basis for potassium/sodium selectivity in the pore of an ion channel," *Plant Physiology*, vol. 132, no. 3, pp. 1353–1361, 2003.
- [153] E. D. Vincill, K. Szczyglowski, and D. M. Roberts, "GmN70 and LjN70. Anion transporters of the symbiosome membrane of nodules with a transport preference for nitrate," *Plant Physiology*, vol. 137, no. 4, pp. 1435–1444, 2005.
- [154] M. A. Piñeros, G. M. A. Cançado, and L. V. Kochian, "Novel properties of the wheat aluminum tolerance organic acid transporter (TaALMT1) revealed by electrophysiological characterization in *Xenopus* oocytes: functional and structural implications," *Plant Physiology*, vol. 147, no. 4, pp. 2131–2146, 2008.
- [155] A. Meyer, S. Eskandari, S. Grallath, and D. Rentsch, "AtGAT1, a high affinity transporter for γ -aminobutyric acid in *Arabidopsis thaliana*," *The Journal of Biological Chemistry*, vol. 281, no. 11, pp. 7197–7204, 2006.
- [156] F. Chaumont, F. Barrieu, R. Jung, and M. J. Chrispeels, "Plasma membrane intrinsic proteins from maize cluster in two sequence subgroups with differential aquaporin activity," *Plant Physiology*, vol. 122, no. 4, pp. 1025–1034, 2000.
- [157] C. Dordas, M. J. Chrispeels, and P. H. Brown, "Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots," *Plant Physiology*, vol. 124, no. 3, pp. 1349–1361, 2000.
- [158] M. Werner, N. Uehlein, P. Proksch, and R. Kaldenhoff, "Characterization of two tomato aquaporins and expression during the incompatible interaction of tomato with the plant parasite *Cuscuta reflexa*," *Planta*, vol. 213, no. 4, pp. 550–555, 2001.
- [159] S. Sakr, G. Alves, R. Morillon, et al., "Plasma membrane aquaporins are involved in winter embolism recovery in walnut tree," *Plant Physiology*, vol. 133, no. 2, pp. 630–641, 2003.
- [160] L.-H. Liu, U. Ludewig, B. Gassert, W. B. Frommer, and N. von Wirén, "Urea transport by nitrogen-regulated tonoplast intrinsic proteins in *Arabidopsis*," *Plant Physiology*, vol. 133, no. 3, pp. 1220–1228, 2003.
- [161] D. Loqué, U. Ludewig, L. Yuan, and N. von Wirén, "Tonoplast intrinsic proteins AtTIP2;1 and AtTIP2;3 facilitate NH_3 transport into the vacuole," *Plant Physiology*, vol. 137, no. 2, pp. 671–680, 2005.
- [162] R. Vera-Estrella, B. J. Barkla, H. J. Bohnert, and O. Pantoja, "Novel regulation of aquaporins during osmotic stress," *Plant Physiology*, vol. 135, no. 4, pp. 2318–2329, 2004.
- [163] W. Wei, E. Alexandersson, D. Golldack, A. J. Miller, P. O. Kjellbom, and W. Fricke, "HvPIP1;6, a barley (*Hordeum vulgare* L.) plasma membrane water channel particularly expressed in growing compared with non-growing leaf tissues," *Plant and Cell Physiology*, vol. 48, no. 8, pp. 1132–1147, 2007.
- [164] W. Lin, Y. Peng, G. Li, et al., "Isolation and functional characterization of PgTIP1, a hormone-autotrophic cells-specific tonoplast aquaporin in ginseng," *Journal of Experimental Botany*, vol. 58, no. 5, pp. 947–956, 2007.
- [165] W.-G. Choi and D. M. Roberts, "Arabidopsis NIP2;1, a major intrinsic protein transporter of lactic acid induced by anoxic stress," *The Journal of Biological Chemistry*, vol. 282, no. 33, pp. 24209–24218, 2007.
- [166] M. Mahdieh, A. Mostajeran, T. Horie, and M. Katsuhara, "Drought stress alters water relations and expression of PIP-type aquaporin genes in *Nicotiana tabacum* plants," *Plant and Cell Physiology*, vol. 49, no. 5, pp. 801–813, 2008.
- [167] N. Shitan, I. Bazin, K. Dan, et al., "Involvement of CjMDR1, a plant multidrugresistance-type ATP-binding cassette protein, in alkaloid transport in *Coptis japonica*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 2, pp. 751–756, 2003.
- [168] S. Gustavsson, A.-S. Lebrun, K. Nordén, F. Chaumont, and U. Johanson, "A novel plant major intrinsic protein in *Physcomitrella patens* most similar to bacterial glycerol channels," *Plant Physiology*, vol. 139, no. 1, pp. 287–295, 2005.
- [169] A.-G. Desbrosses-Fonrouge, K. Voigt, A. Schröder, S. Arrivault, S. Thomine, and U. Krämer, "Arabidopsis thaliana MTP1 is a Zn transporter in the vacuolar membrane which mediates Zn detoxification and drives leaf Zn accumulation," *FEBS Letters*, vol. 579, no. 19, pp. 4165–4174, 2005.
- [170] D. Becker, D. Geiger, M. Dunkel, et al., "AtTPK4, an *Arabidopsis* tandem-pore K^+ channel, poised to control the pollen membrane voltage in a pH- and Ca^{2+} -dependent manner," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 44, pp. 15621–15626, 2004.
- [171] T. P. Durrett, W. Gassmann, and E. E. Rogers, "The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation," *Plant Physiology*, vol. 144, no. 1, pp. 197–205, 2007.