Plant biomass increase: recent advances in genetic engineering

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Plant biomass enhancement using transgenesis in model as well as in crop plants both under optimal and stress controlled (greenhouse or growth chamber) and field conditions is observed. It was documented that genetic engineering approaches allow reaching up to two-fold increase of biomass in optimal conditions. Both transgenic and initial plants reduce biomass production under stress. It was demonstrated that it can be up to 2-fold increase in productivity of transgenic plant versus untransformed one in stress. Some heterologous gene expression features influencing plant biomass production are discussed.

Keywords: biomass, dry weight, fresh weight, stress, transgenic plants.

Introduction. Plants are photoautotrophic organisms which using light energy can transform inorganic carbon dioxide to carbohydrates and then to other compounds and in this manner form their biomass. We use plants for food and in different fields of our activity such as construction, textile, pharmaceutical and chemical industries. Plant biomass has also been considered as an important renewable source of biofuels [1, 2]. Enhancing plant height, growth rates and total biomass retains a principal demand due to the growing of the world population.

Considerable efforts of the plant scientists have been aimed at the problem of plant biomass increase. Plant growth markedly depends on availability of essential nutrients such as nitrogen and phosphorus which modulate a number of aspects of the plant development including root and shoot branching and enlargement, leaf growth, flowering time, and regulate expression of many genes involved in nitrogen and carbon metabolism [3, 4]. Genetic engineering investigations on nitrogen assimilation for the improvement of nitrogen efficiency have recently been deeply reviewed [5, 6].

Engineering of photosynthesis in crop species can significantly increase agricultural productivity. Summarizing the results obtained in the model objects on improvement of photosynthetic electron transport chain, rubisco activity and specificity, Rubisco activation state, photorespiration, sedoheptulose-1,7-bisphosphatase/fructose-1,6-bisphosphatase activity, Peterhansel *et al*. focused their attention on necessity to test elaborated approaches in true crops under conditions of agricultural production [7].

The regulation of carbon allocation between photosynthetic source – leaves and sink tissues – is an important factor controlling plant yield [8]. Plant organs are initiated as primordial outgrowths, and require controlled cell division and differentiation to achieve their final size and shape. Plant hormones thinly regulate plant growth and affect total biomass and yield production [9–12]. Transgenesis allows identification of the mechanisms of complex resistance to stresses and thus leads to an increase in production of plant biomass in non-optimal conditions [13].

The aim of this review was to show the last achievements in genetic engineering and perspectives of some approaches (different promoters, fused genes) for opti-

mization of ectopic gene expression for crop biomass increase under field.

Biomass increase in optimal growth conditions. Changes in the plant biomass accumulation can be achieved by regulation of the carbohydrate metabolism [14–16]. Simultaneous upregulation of UDP-glucose pyrophosphorylase, sucrose synthase and sucrose phosphate synthase resulted in enhanced primary growth; for example, for tobacco *Nicotiana tabacum* L. in some cases an increase in height growth was over 50 % [17]. Due to the increased sucrose phosphate synthase activity the total *Arabidopsis* (*Arabidopsis thaliana* L.) biomass was 2-fold higher in *AtPAP2* (purple acid phosphatase) overexpressed plants than in wild-type [18]. The RNAi-mediated down-regulation of glucan water-dikinase (the primary enzyme required for starch phosphorylation) under the control of an endosperm-specific promoter led to a decrease in starch phosphate content in common wheat (*Triticum aestivum* L.) [19]. Consistent increase in vegetative biomass, grain size and grain yield was observed in subsequent generations in both greenhouse and field trials. Overexpression of sucrose synthase (*SusA1*) gene from a superior quality fiber germplasm line 7235 in cotton *Gossypium hirsutum* increased fiber length and strength [20]. Increasing *GhSusA1* transcript abundance in vegetative tissues led to elevated seedling biomass.

Many biotechnological systems have been established to advance enhancing plant height, growth rates and total biomass with emphasis on increasing the concentration of the plant hormones or on their signalling. Overexpression of the most studied gibberellin biosynthesis enzyme, GA 20-oxidase gene, resulted in an excessively high activity of gibberellin deactivating enzyme, GA 2-oxidase. It was shown that silencing the gibberellin deactivating enzyme in tobacco plants resulted in a dramatic improvement of their growth characteristics, compared with the wild type and GA 20-oxidase over-expressing plants [21]. Suppression of *PtGA2ox4* and *PtGA2ox5* genes belonging to the poplar C19 gibberellin 2-oxidase (GA2ox) gene subfamily which primarily expressed in aerial organs led to significant increase of leaf size (+ 50 %), stem height (+ 20 %), diameter (+ 10 %) and biomass (+ 30 %) in *Populus*. It had no effect on the root development [22]. Transplastomic tobacco plants expressing β-glucosidase (*Bgl-1*),

as compared with untransformed plants, have shown 2-fold higher gibberellin (of both GA1 and GA4 levels) in leaves but not in other organs. The elevated levels of other plant hormones, including zeatin and indole-3-acetic acid, are observed in BGL-1 lines. These plants flowered 1 month earlier with an increase in biomass (1.9-fold), height (1.5-fold), and leaf area (1.6-fold) in comparison with untransformed plants [13].

The increase in organ size due to enhanced cell proliferation, without contribution from cell expansion was observed in *Arabidopsis* and tobacco plants expressing *ARGOS* (full-length cDNA from Chinese cabbage and *A. thaliana* leaves, respectively) gene [23, 24].

Significant axillary bud outgrowth at all nodes on the main stem with the pronounced branch development from the more basal nodes was observed in narrow-leafed lupin (*Lupinus angustifolius* L. cv Merrit) transgenic lines with isopentenyl transferase (*ipt*) gene from *Agrobacterium tumefaciens* coupled to a flower-specific promoter (*TP12*) from *N. tabacum* [25]. IPT expression increased cytokinin levels in flowers, meristem tissues and phloem exudates in a form specific manner. The total number of pod set in some transgenic lines was increased and grain size was not significantly altered compared to control. In transgenic tobacco expressing fused *AOC-ipt* gene, cytokinins increased only two to three fold, and the plants grew more vigorously than the *AOC* (the allene oxide cyclase gene from the salt-tolerant plant *Bruguiera sexangula*, which displays salt tolerance) transgenic plants lacking the *ipt* gene [26]. It was reflected in rapid plant growth, longer flowering period, greater number of flowers, more seed product, and increased chlorophyll synthesis. Total dry weight increased in *AOC-ipt* tobacco up to 1.27-fold in comparison with control ones and retained at the control level in *AOC* plants in nonstress greenhouse conditions.

In our experiments *cyp11A1* canola (*Brassica napus* L.) plants expressing cytochrome $P450_{SCC}$ from bovine adrenal cortex mitochondria produced increased fresh weight (+ 33 %) and total soluble proteins (+ 71 %) in comparison with the control ones in non-stress aseptic conditions [27]. In animals cytochrome $P450_{SCC}$ catalyzes cholesterol oxidation with formation of pregnenolone [28]. Superoxide radicals are formed during these reactions. We detected SOD activity increase in leaves

of some *cyp11A1* canola plants in optimal growth conditions when it was compared with untransformed ones.

The enhanced initial seminal root growth in transgenic rice (*Oryza sativa* L.) seedlings expressing sheep serotonin *N*-acetyltransferase (*NAT*) matched their increased root biomass [29]. *NAT* is believed to be a rate-limiting enzyme in the melatonin biosynthesis in animals. It has previously been shown that exogenous melatonin application enhances plant growth [30]. Among a number of actions, melatonin is a direct free radical scavenger and an indirect antioxidant [31].

Agrobacterium rhyzogenes-mediated transformation technique can be used for the plant biomass improvement. The increased dry weight was observed in *rolA*-transformed blue grama grass (*Bouteloua gracilis* (H. B. K.) Lag. ex Steud.) plants under greenhouse conditions, it was mainly related to shoot growth [32]. Several liquorice *Glycyrrhiza glabra* L. hairy root clones bearing *rolB* gene were more branched and showed about 8 fold higher root biomass on solid medium than untransformed ones [33].

Arabidopsis lines overexpressing *pdx2* gene which is involved in the *de novo* vitamin B6 biosynthesis pathway in plants have considerably larger vegetative and floral organs and it is related to a general increase in total protein, lipid and carbohydrate content [34].

The STOREKEEPER (STK) family of DNA-binding proteins works as transcription factors and the ectopic expression of two *stk*-like genes from *Arabidopsis*, *stk01* and *stk03*, in tobacco increased the number of vegetative internodes and promoted plant and leaf size, stem diameter and sturdiness [35].

Selective genes neither created significant unintended pleiotropic effects on gene expression nor led to increased biomass formation [36, 37]. In some experiments the plants bearing vector with only selective gene were used as control ones. Any observations about biomass increase were fixed ([13] – *nptII* gene,[38] – *bar* gene). We did not also detect differences in biomass production between untransformed and transgenic canola plants simultaneously expressing *nptII* and promotorless *bar* genes [39].

Genetic engineering approaches allow reaching more than 2-fold biomass increase in model as well as in crop plants under both optimal controlled (greenhouse or growth chamber) and field conditions (Table 1).

Advantages under stress conditions. The expression of majority of genes which are mentioned in this chapter gives the advantages for transgenic plants under stress growth. These plants often do not differ from control ones in the optimal conditions. Both transgenic and initial plants reduce biomass production under stress influences. Transgenesis leads to new mechanisms of complex resistance to stresses of different origin and this knowledge provides creating the plants with high productivity under unfavorable conditions.

The rate of photosynthesis declines at moderately high temperatures. This can be attributed to a reduced ability of Rubisco activase to achieve optimum activation of Rubisco, which causes lower Rubisco activity. Transgenic *Arabidopsis* lines expressing chimeric activase (Rubisco recognition domain in more thermostable tobacco activase was replaced with that from *Arabidopsis*) showed higher rates of photosynthesis than the wild type after a short exposure to higher temperatures [44]. They also formed higher biomass and seed yield when compared with the wild type plants exposed to moderately elevated temperature.

Tobacco chloroplast engineering of β-alanine pathway by over-expression of the *Escherichia coli panD* gene, which catalyzed decarboxylation of l-aspartate to generate β-alanine and carbon dioxide, enhances thermotolerance of photosynthesis and biomass production following high temperature stress [45].

Limited availability of phosphate ion (P_i) and nitrogen reduces plant growth in natural ecosystems. Compared with wild-type plants, the transgenic canola over-expressed barley alanine aminotransferase (*AlaAT*) driven by canola root specific promoter (*btg26*), had increased biomass (FW 1.98 fold, DW 1.75 fold) and seed yield in both laboratory and field under low nitrogen conditions, whereas no differences were observed under high nitrogen [46]. These changes resulted in a 40 % decrease in the amount of applied nitrogen fertilizer required under field conditions to achieve the yields equivalent to wild-type plants.

Phosphorus (P) is an essential nutrient for the plant growth and development, but is generally unavailable and inaccessible in soil, since applied P is mostly fixed to aluminium (Al) and ferrum (Fe) in acidic soils and to calcium (Ca) in alkaline soils. Increased organic acid excretion is one of the mechanisms by which plants use

Table 1
Biomass increase in transgenic plants under normal growth conditions

Species	Gene(s)	Increase, up fold	References
Arabidopsis	The overexpression of **AtPAP2**, a purple acid phosphatase	2 (total FW)	[18]
	Full-length cDNA from Chinese cabbage leaves, **BrARGOS**	2.2 (leaf FW)	[23]
Tobacco (*Nicotiana tabacum*)	Simultaneous UDP-glucose pyrophosphorylase (**UGPase**), sucrose synthase (**SuSy**) and sucrose phosphate synthase (**SPS**) overexpression	1.5 (height growth)	[17]
	Isopentenyl transferase gene (**ipt**) downstream transcriptionally fused with **AOC** (allene oxide cyclase gene from the salt-tolerant plant *Bruguiera sexangula*, which displays salt tolerance) gene (**AOC-ipt**)	1.27 (total DW)	[26]
Transplastomic tobacco (*Nicotiana tabacum* L.)	β-glucosidase (**Bgl-1**)	1.9 (total FW); 1.5 (height); 1.6 (leaf area)	[13]
Rice *Oryza sativa* L. cv. Taichung 65	Partial suppression of endogenous OsBRI1 (a rice *BRASSINOSTEROID INSENSITIVE1* ortholog)	1.3 (grain yield)	[40]
Rice	Expression of the *Arabidopsis HARDY* (**HRD**) gene	2 (total FW)	[41]
Rice cv. Dongjin	Expression of the sheep serotonin *N*-acetyltransferase (**NAT**)	1.44 (root FW)	[29]
Canola cv. Mariia	**cyp11A1** gene encoding cytochrome $P450_{SCC}$ from bovine adrenal cortex mithochondria	1.33 (total FW); 1.71 (total soluble protein) *in vitro*	[27]
Common wheat (*Triticum aestivum* L.)	RNAi-mediated down-regulation of Glucan, Water-Dikinase (**GWD**), the primary enzyme required for starch phosphorylation, under the control of an endosperm-specific promoter	1.29 (grain yield) in glasshouse pot trials	[19]
Common wheat cv. Hi-Line	**HVA1** gene, encoding a group 3 LEA protein from barley (*Hordeum vulgare* L.)	1.1 (total DW)	[42]
Switchgrass (*Panicum virgatum* L.)	miR156 suppression of SQUAMOSA PROMOTER BINDING PROTEIN LIKE (**SPL**)	1.63–2.01 (total DW)	[43]
Blue grama grass (*Bouteloua gracilis*)	**rolA** gene of *A. rhizogenes*, under the control of a double cauliflower mosaic virus (CaMV) 35S	2 (total DW) under greenhouse conditions	[32]
Liquorice *Glycyrrhiza glabra*	**rolB** gene of *A. rhizogenes*, under the control of *nos* promoter	8 (hairy root FW) *in vitro*	[33]
Poplar (hybrid clone INRA 717-IB4 (*Populus tremula × P. alba*)	Suppression of **PtGA2ox4** and **PtGA2ox5** genes belonging to the poplar C19 gibberellin 2-oxidase (**GA2ox**) gene subfamily	1.5 (leaf size); 1.2 (stem height); 1.1 (stem diameter); 1.3 (leaf FW)	[22]
Cotton *Gossypium hirsutum* L. accession 7235 and TM-1	Overexpression of sucrose synthase (**SusA1**) gene from a superior quality fiber germplasm line 7235 of *Gossypium hirsutum*	1.4 (total DW in both seedling and boll stages of development)	[20]

*FW – fresh weight; DW – dry weight.

to enhance P uptake. Overexpressing the mitochondrial malate dehydrogenase (*MDH*) gene from the mycorrhizal fungi *Penicillium oxalicum* transgenic tobacco lines which showed the highest level of MDH activity and malate exudate were characterized by a significant increase in growth over wild-type [47]. The construction of a new citrate synthesis pathway by simultaneous overexpression of own citrate synthase and a mutant (with reduced sensitivity to organic acid inhibition) phosphoenolpyruvate carboxylase from *Synechococ-*
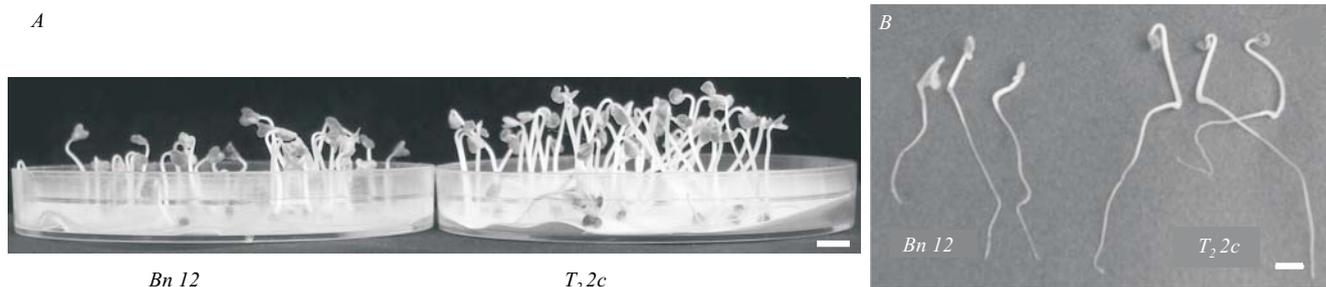
Control (Bn12) and *cyp11A1* expressing cytochrome P450$_{scc}$ from bovine adrenal cortex mitochondria (T$_2$2c) seedling growth under high temperature (26 °C, thermostat): *A* – forth germination day; *B* – third germination day. Scale bars: 1 cm

cus vulcanus in the cytoplasm of transgenic tobacco leaves led to an enhanced Al resistance in plants [48].

The biomass of several *MtPHY1* (phytase, under control of the root-specific MtPHY1 promoter) alfalfa (*Medicago sativa* L.) lines was three times that of the control when plants were grown in sand supplied with phytate as the sole P source and two times when the plants were grown in natural soils without additional P supplement [49]. Over-expression of *GmEXPB2* (a soybean b-expansin) gene resulted in 28 % increase in soybean (*Glycine max* (L.) Merr.) fresh weight at low P [50].

Protein dephosphorylation mediated by protein phosphatases plays a major role in signal transduction of plant responses to environmental stresses. The fresh weight increase in transgenic *Arabidopsis* lines bearing *Faseolus vulgaris* phosphatase (PvPs2:1) was significantly larger than that in wild type plants at low and high P levels, especially at the latter, accompanying by the upper of total P content and total root length [51]. *AtPAP18* (*Arabidopsis* purple acid phosphatase encoding gene) tobacco plants exhibited significant increase in the acid phosphatase activity leading to an improved biomass production in both P$_i$-deficient and P$_i$-sufficient conditions [52].

Transgenic canola, expressing the gene for the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and/or untransformed canola treated with *Pseudomonas putida* UW4, which expressed the same gene, had greater shoot biomass compared to the untransformed canola under the low flood-stress conditions [53].

Inositol polyphosphate kinase (IPK) participates in inositol metabolism, calcium signaling, stress response, gene transcription and other physiological and biochemical processes. Transgenic soybean plants with the *ThIPK2* gene (*IPK* from *Thellungiella halophila*) dis-

played water deficit-, salt- and oxidative-tolerance compared to untransformed controls [54]. Furthermore, the expression of *ThIPK2* altered the ratio of fatty acid components in soybean seeds, resulting in an increase of oleic acid (C18:1). The seed size was also increased in the transgenic plants.

The ectopic antioxidant gene expression leads to an increased plant resistance to stress of different origin that is accompanied by the formation of a larger plant biomass. The first enzyme in the detoxifying process of reactive oxygen species which were generated as by-products of fatty acid β-oxidation, photorespiration, photosynthesis, and environmental conditions such as extreme temperatures and/or water stress, especially in combination with high light intensities is superoxide dismutase (SOD). It converts superoxide radicals to hydrogen peroxide. Some independent transgenic alfalfa (*M. sativa*) plants heterologously expressing Mn-SOD in mitochondria or chloroplasts had twice the herbage yield of the control plants after one winter period of growth [55]. The transgenic canola which overexpressed wheat Mn SOD3.1 produced more vigorous seedlings under both normal and stress conditions [56].

In our experiments *cyp11A1* canola plants expressing cytochrome P450$_{scc}$ from bovine adrenal cortex mitochondria formed larger biomass during different ontogenetic stages compared with untransformed control [27, 57]. Some of transgenic lines formed seedlings with longer roots (by 50 %) and hypocotyles (by 85 %) during germination at high temperature (Figure) [57].

Dehydroascorbate reductase (DHAR) maintains redox pools of ascorbate (AsA) by recycling oxidized AsA to reduced AsA. *OsDHAR1* rice significantly increased photosynthetic capacity and antioxidant enzyme

activities under paddy field conditions [58]. It also imp-roved grain yield and biomass due to the increase of culm and root weights and enhanced panicle and spi-kelet numbers.

Ascorbate oxidase is an apoplastic enzyme, which also controls the redox ascorbate pool. Tomato *Sola-num lycopersicum* plants with decreased ascorbate oxi-dase activity due to RNA-interference formed a larger fruit yield under limited water and leaf removing [59]. The ascorbate oxidase plants also showed increase in stomatal conductance and leaf and fruit sugar content, as well as an altered apoplastic hexose:sucrose ratio.

Glycine betaine is an osmoprotectant that plays an important role and accumulates rapidly in many plants during salinity or drought stress. Choline monooxyge-nase (CMO) is a major catalyst in the synthesis of gly-cine betaine. The seed cotton yield of the *AhCMO* (cho-line monooxygenase from *Atriplex hortensis*) plants was lower under normal conditions, but was significantly higher than that of non-transgenic plants under the salt-stressed field conditions [60]. Common wheat lines that were transgenic for the *betA* gene encoding choline de-hydrogenase from *E. coli* were less injured and exhibi-ted greater root length and growth compared with the wild type under drought stress [61].

Glutathione (GSH), a low-molecular-weight tripep-tide molecule, that plays an important role in cell func-tion and metabolism as an antioxidant, is synthesized by γ-glutamylcysteine synthetase (γ-ECS) and glutathi-one synthetase (GS). The transgenic rice plants expres-sing the *ECS* gene from *Brassica juncea* L. under the re-gulation of a stress-inducible *Rab21* promoter display-ed a moderate increase in biomass (up to 1.2 times in total FW and 1.1 fold in root FW) and rice grain yield (up 1.2 fold in total seed weight) under general paddy field conditions [62].

AlSAP (stress-associated protein from the halophy-te grass *Aeluropus littoralis*) durum wheat (*Triticum du-rum*) of the commercial cv. Karim exhibited improved germination rates and biomass production under sali-nity and osmotic stress conditions [63]. Following long-term salt or drought stress greenhouse trials, *AlSAP* lines produced normally filled grains whereas wild-ty-pe plants either died at the vegetative stage under the salt stress or showed markedly reduced grain filling un-der the drought stress.

Isopentenyltransferase (IPT) is a critical enzyme in the cytokinin biosynthetic pathway. The *ipt* expression under the control of a maturation- and stress-induced SARK promoter delayed stress-induced plant senescen-ce that resulted in an enhanced drought tolerance in peanut (*Arachis hypogaea* L.) in both laboratory and fi-eld conditions [64]. The transgenic peanut plants main-tained 2 fold higher photosynthetic rates and transpira-tion than wild-type plants under the reduced irrigation conditions. More importantly, the *ipt* peanut plants pro-duced significantly higher yields than wild-type plants in the field dryland (50 and 29 g/plant, respectively) while at high irrigation no significant yield differencies were observed between the transgenic and initial plants (42 and 40 g/plant, respectively).

The *AOC* gene plays a role in salt tolerance. When the *ipt* gene transcriptionally fused with *AOC* in the frame of *AOC-ipt*, slight cytokinin increases were obtained in these transgenic plants which played a positive role in im-provement of plant growth. Dry weight was increased in the *pVKH35S-AOC* and *pVKH35S-AOC-ipt* up to 1.12 times and 1.39 fold, respectively, in comparison with the control plants under drought in a greenhouse. It was more prominent under high salinity when plants were watered by sea water and increase in the plant dry weight reached up to 1.59 and 1.37 times (*pVKH35S-AOC-ipt* and *pVKH35S-AOC*, respectively) [26].

As an innate and adaptive response to water deficit, land plants avoid potential damage by rapid biosynthe-sis of the abscisic acid (ABA), which triggers stomatal closure to reduce transpirational water loss. Recent ge-netic studies have pinpointed protein farnesyltransfe-rase as a key negative regulator controlling ABA sensi-tivity in the guard cells. Conditional and specific down-regulation of farnesyltransferase in canola using the *Arabidopsis* hydroxypyruvate reductase promoter dri-ving an RNAi construct resulted in yield protection against drought stress in the field [10]. There was no sig-nificant difference in growth and agronomic performan-ce between the genetically engineered transgenic ca-nola and its wild-type control in optimal conditions. However, under the moderate drought stress conditions at flowering, the transgenic plants produced significant-ly higher seed yield.

High ectopic expression of the tomato abscisic acid-induced myb1 (*SlAIM1*) gene encoding the R2R3MYB

Table 2
Transgenic plant advantages in biomass production in different stress conditions

Species	Gene(s)	Conditions	Increase, up fold	References
Arabidopsis *Arabidopsis thaliana* L.	*Faseolus vulgaris* phosphatase (***PvPs*2:1**)	High P level	1.2 (total FW)	[51]
Tobacco *Nicotiana tabacum* L.	Arabidopsis purple acid phosphatase encoding gene, ***AtPAP18***	In both P_i-deficient and P_i-sufficient conditions	1.41 (total FW)	[52]
	Mitochondrial malate dehydrogenase (***MDH***) gene from the mycorrhizal fungi *Penicillium oxalicum*	Al-phosphate medium	1.49 (total FW)	[47]
	The same	Fe-phosphate medium	1.29 (total FW)	[47]
	The same	Ca-phosphate medium	1.28 (total FW)	[47]
	The *Escherichia coli* l-aspartate-α-decarboxylase (AspDC) encoded by the ***panD*** gene	High temperature	1.3–1.4 (total FW)	[47]
	1) Phosphoenolpyruvate carboxylase (***pepc***); 2) citrate synthase (***cs***); 3) double transformants	Al stress	1) 1.2; 2) 1.49; 3) 1.82 (height); 1) 1.33; 2) 2.55; 3) 3.73 (root DW)	[48]
	AOC-ipt-isopentenyl transferase gene (***ipt***) downstream transcriptionally fused with ***AOC*** (allene oxide cyclase gene from *Bruguiera sexangula*, which displays salt tolerance) gene	Drought stress	1.39 (total DW)	[26]
Alfalfa cv. Regen SY-4D	Phytase (***MtPHY1***) gene from *Medicago truncatula* under the root-specific MtPT1 promoter	In natural soils without additional Phosphorus supplement	2 (total FW)	[49]
	The same	With phytate as the sole P source	3 (total FW)	[49]
Alfalfa	***Mn-SOD*** in mitochondria or chloroplast	Cold stress	2 (total FW)	[49]
Rice cv. Ilmi	*Brassica juncea* γ-glutamylcysteine synthetase (***BrECS***) gene under the regulation of a stress-inducible Rab21 promoter	Osmotic stress (100 mM NaCl)	1.2 (total FW); 1.1 (root FW); 1.2 (total seed weight)	[62]
Rice cv. Nipponbare	Arabidopsis *HARDY* (***HRD***) gene, an AP2/ERF-like transcription factor	Drought stress	1.5 (total FW) in greenhouse	[41]
Durum wheat (*Triticum durum*) commercial cv. Karim	***AlSAP*** (stress-associated protein) gene from the halophyte grass *Aeluropus littoralis*	Osmotic stress (50 mM NaCl)	3 (leaf DW)	[63]
	The same	Osmotic stress (150 mM NaCl)	4 (leaf DW)	[63]
Common wheat (*Triticum aestivum* L.) cv. Jinan 17	***betA*** encoding choline dehydrogenase from *Escherichia coli*	Drought stress	1.36 (total DW)	[61]
Canola cv. Westar	Barley alanine aminotransferase (***AlaAT***) under canola root specific promoter (***btg26***)	Low nitrogen	1.98 (total FW); 1.75 (total DW)	[46]
	1-Aminocyclopropane-1-carboxylate (***ACC***) deaminase from *Pseudomonas putida* UW4 under control of the *rolD* promoter from *Agrobacterium rhizogenes*	Low flood stress	1.34 (total DW) in field conditions	[53]

Conclusions of Table 2

Species	Gene(s)	Conditions	Increase, up fold	References
Canola cv. Westar	1-Aminocyclopropane-1-carboxylate (**ACC**) deaminase + *Pseudomonas putida* UW4, which also expresses ACC deaminase	Low flood stress	1.38 (control total DW)–1.31 (transgenic total DW) in field conditions	[53]
Canola doubled haploid line DH-12075	Wheat mitochondrial Mn superoxide dismutase (**Mn SOD3.1**)	Cold, drought, high temperature (field and *in vitro*)	1.4 (total FW)	[56]
Canola cv. Mariia	**cyp11A1** gene encoding cytochrome P450$_{SCC}$ from bovine adrenal cortex mithochondria	Osmotic stress (500 mM mannitol) *in vitro*	2 (total FW)	[27]
	The same	High temperature	1.31 (total FW)	[57]
Canola cv. Youyan N 9	*Brassica napus* heme oxygenase (**BHO-1**)	Hg stress	1.41–1.50 (total DW)	[69]
Tomato (*Solanum lycopersicum*) cvs CastlemartII and Micro-Tom	The tomato abscisic acid-induced *myb1* (**SlAIM1**) gene encoding an R2R3MYB transcription factor	Osmotic stress (200–250 mM NaCl)	1.1 (total FW)	[65]
Sugar beet (*Beta vulgaris* L.) cv. Heitian N 1	An *Arabidopsis thaliana* vacuolar Na^+/H^+ antiporter gene, **AtNHX3**	Osmotic stress (500 mM NaCl)	2 (total DW); 2.2 (total FW); 2.2 (storage root DW)	[70]
Soybean (*Glycine max* (L.) Merr.) cv. HN89	A soybean β-expansin (**GmEXPB2**) gene	Low P level	1.28 (total FW)	[50]
Peanut (*Arachis hypogaea* L.) New Mexico Valencia A	Isopentenyltransferase (**IPT**) under the control of a maturation- and stress-induced promoter (P$_{sark}$)	Drought stress	1.84–1.61 (shoot FW-DW); 2.88–2.24 (root FW-DW) in growth chamber	[64]

transcription factor resulted in reduced plant growth compared with the control plants under normal growth conditions [65].

However, the 35S:SlAIM1 plants grow significantly better than the wild-type controls under high salinity, and only marginally lower than the 35S: SlAIM1 plants grown without salt stress.

Different plants exhibit different, sometimes opposite, response to the same foreign gene introduction. Expression of Δ^1-pyrroline-5-carboxylate synthetase (P5CS) from mothbean (*Vigna aconitifolia* L.) under the control of a stress-inducible promoter led to stress-induced overproduction of the P5CS enzyme and proline accumulation in the transgenic rice plants [66]. Second-generation plants showed an increase in biomass under salt- and water-stress conditions as compared to the untransformed plants. But biomass increase in chickpea (*Cicer arietinum* L.), which accumulated proline (up to 6-fold) due to mutagenized *P5CS* expression, was not documented [67]. Authors supposed that the enhanced proline level had little bearing on the components of yield architecture that are significant in overcoming the negative effects of drought stress in chickpea.

The transformation of dicots with the *rolA* gene results in the shortened internode length, green darkened leaves, reduced apical dominance, leaf wrinkling, decreased length-to-width leaf ratio, shortened styles, larger flower size, reduced flower number, condensed inflorescences, male sterility, and retarded onset of flowering [68]. The *rolA B. gracilis* (the important forage grass) lines showed a 2-fold reduced root system, but they produced up to twice as much foliage as control plants due to enhanced height (up 1.3 fold) and increased tillering and leaf number (up to 1.7 and 1.5 fold, respectively) [32].

Transgenesis allows reach up to 2 fold biomass increase versus the untransformed (wild type or tissue culture) plants in stress (Table 2).

Conclusions. Genetic engineering approaches allow reaching more than 2-fold biomass increase in model as well as in crop plants under optimal conditions. Transgenesis can reveal the new mechanisms of complex resistance to stresses of different origin and this knowledge may be used to create plants with high productivity under unfavorable conditions. It leads to reduced biomass losses under stress nearly 2-fold. Overexpression of some genes of phosphorus and nitrogen metabolism offers an effective approach for reducing the consumption of chemical fertilizers through increased acquisition of soil nutrients and mobilization of internal resources. In recent years the significant amount of studies have been focused at crops such as canola, rice and wheat under field conditions. It should facilitate the introduction of these developments in the agriculture practice.

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Збільшення біомаси рослин: останні досягнення
генетичної інженерії

Резюме

Вирішення завдань збільшення біомаси рослин з використанням трансгенезу роглянуто як на модельних об'єктах, так і на прикладах сільськогосподарських культур, вирощуваних за оптимальних і контрольованих стресових (теплиця або клімакамера) та польових умов. Показано, що генно-інженерні підходи дозволяють досягти подвоєння біомаси за оптимальних умов. І трансгенні, і вихідні рослини зменшують накопичення біомаси внаслідок дії стресових факторів. Продемонстровано, що трансгенні рослини здатні збільшувати свою продуктивність до двох разів порівняно з нетрансформованими за умов стресу. Обговорюються деякі особливості гетерологічної експресії генів, які впливають на накопичення біомаси.

Ключові слова: біомаса, стрес, суха маса, сира маса, трансгенні рослини.

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Увеличение биомассы растений: последние
достижения генетической инженерии

Резюме

Решение задачи увеличения биомассы растений с использованием трансгенеза рассмотрено как на модельных объектах, так и на примерах сельскохозяйственных культур, выращиваемых в оптимальных и контролируемых стрессовых (теплица или климакамера), а также полевых условиях. Показано, что генно-инженерные подходы позволяют достичь удвоения биомассы при оптимальных условиях. И трансгенные, и исходные растения уменьшают накопление биомассы в результате действия стрессовых факторов. Продемонстрировано, что в условиях стресса трансгенные растения способны увеличивать свою продуктивность до двух раз по сравнению с нетрансформированными. Обсуждаются некоторые особенности гетерологической экспрессии генов, влияющие на накопление биомассы.

Ключевые слова: биомасса, стресс, сухая масса, сырая масса, трансгенные растения.

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