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# ALTERATIONS IN SUPEROXIDE DISMUTASE ACTIVITY DUE TO HETEROLOGOUS GENE EXPRESSION: PLANT PECULIARITIES

Plant cells are continuously exposed to reactive oxygen species (ROS) generated as by-products of photosynthesis, photorespiration, and fatty acid в-oxidation. Environmental conditions such as extreme temperatures and/or water stress, especially in combination with high light intensities, and some pathogens can cause oxidative stress damage by overproduction of ROS. The first enzyme in the detoxifying process is superoxide dismutase (SOD, EC 1.15.1.1). Originally discovered by McCord & Fridovich in 1969 [1], it reacts with superoxide radicals to produce hydrogen peroxide and oxygen.

Three SOD isozymes are reported in plants. There are copper/zinc SOD (Cu/ZnSOD), manganese SOD (MnSOD), and iron SOD (FeSOD) [2]. Number of SOD izoforms is species specific [3]. Different SOD izoforms differently participate in various developmental processes and unfavourable conditions [4].

To investigate the influences of SOD activity changes on plant features, the transgenic plants with up- and downregulated *SOD* expression have been produced (table). The SOD activity decrease was studied in *Arabidopsis thaliana* plants when expression of their own *MnSOD* was suppressed by antisense [5]. Constitutive expression of an antisense construct allowed select two lines which possessed an 80 % decrease in the *MnSOD* transcript level leading to a 70 % and 60 % decrease in MnSOD protein level, respectively. Root growth of these plants was reduced up to 1.4-fold by the lower MnSOD protein level, even under non-stressed conditions. Shoot seedling growth was not obviously affected. Soil-grown plants were characterized by late flowering phenotype. They increased biomass due to prolongation of vegetative growth. The higher ascorbate and glutathione peroxidise activities, increased FeSOD levels, and an increased pool size of the redox buffers glutathione (by 50 %) and ascorbate (by 43 %) were detected. Authors speculated that overcompensation of defects in antioxidant enzymes seems to be a general response to the loss of antioxidant enzymes.

Physiological and biochemical characterization of knockdown chloroplast-Cu/ZnSOD (KD-SOD) Arabidopsis plants revealed they had a decreased rate of photosynthesis and lower level of chlorophyll [6]. They were profoundly suppressed in their growth and development. However, the content of oxidized proteins, a measure of oxidative stress, was not significantly different between KD-SOD and wild type plants. *In situ* staining for superoxide did not document a difference between KD-SOD and wild type plants grown under controlled conditions. KD-SOD plants were delayed by at least 3 days in their flowering; however, they produced fertile seeds [6]. Knockout Arabidopsis FeSOD2- and FeSOD3-deficient mutants had pale green phenotypes on agar plates, and double mutants had the severe albino ones. Chloroplast development was arrested in young seedlings of the double mutants. These plants were highly sensitive to high and low light intensity and developed increased levels of ROS during extended darkness [7].

The MnSOD-deficient and FeSOD-deficient bean (*Phaseolus vulgaris* L.) mutants ($M_3$) manifested normal growth due to Cu/ZnSOD upregulation in seedlings and mature plants. The arsenic-treated mutants showed normal growth, and no significant accumulation of ROS was observed in leaf as revealed by ROS-imaging study. In double mutants, total absence of both MnSOD and FeSOD transcripts was accompanied by significant down-regulation of Cu/ZnSODs, resulting in ROS accumulation at high magnitude and appearance of necrotic spots on photosynthetic organs both in normal conditions and during arsenic exposure [8].

To obtain plants with improved tolerance to environmental stresses, the tremendous efforts have been making for heterologous *SOD* and other gene overexpression by leading research groups around the world [9 and references herein]. These plants had often some advantages under growth in favourable conditions compared initial plants as well (table).

## Conclusions

Transgenic plants having increased SOD activity would demonstrate no phenotype changes under favourable conditions [12, 14, 16, 19, 21, 24, 34] (or these changes have not been studied

*Table*

**Plants possessing elevated SOD activity under non-stress conditions due to heterologous gene expression**

| Target plant | Gene | Gene resource | Total SOD activity increase*, up | Improved tolerance to stress, kind | Phenotype compared with control under favourable conditions | Ref. |
|---|---|---|---|---|---|---|
| Canola *Brassica napus,* cv Westar | *MnSOD* | Triticum aestivum | 2.5 | Aluminium stress; methyl viologen | Not studied | [10] |
| Canola doubled haploid line DH-12075 | *MnSOD* | Triticum aestivum | 1.4 | Cold, drought, high temperature (in both field and *in vitro*) | Higher biomass production and vegetative growth rate, faster flowering | [11] |
| Tobacco *N. tabacum* cv. PBD6 | *Mn-SOD* | Nicotiana plumbaginifolia | 2-4 – in chloroplasts 8 – in mitochondria | Ozone damage (due to expression in chloroplasts only) | No phenotype changes | [12] |
| Tomato *Lycopersicon esculentum* cv. Zhongshu No. 5 | *Mn-SOD* | Hevea brasiliensis | 2 | NaCl, methyl viologen | Not studied | [13] |
| Rice *Oryza sativa* L. | *Mn-SOD* expressed in chloroplasts due to targeting | yeast | 1.7 | NaCl, methyl viologen | No phenotype changes | [14] |
| Alfalfa *Medicago sativa* L. clones N4 and S4 | *Mn-SOD* | | 2 | Winter survival | Up to 2-fold higher biomass production under field trials | [15] |
| Poplar *Populus davidiana* x *P. bolleana* | *MnSOD* | Tamarix androssowii | 1.3 | NaCl | Relative both MDA content and electroconductivity are lower. Phenotypically normal | [16] |
| Petunia *Petunia hybrida* | *MnSOD* | | N.c. | Methyl viologen, NaCl, cold | Higher biomass production, changes in flower size | [17–18] |
| Arabidopsis thaliana | Cu/ZnSOD | Potentilla atrosanguinea | 1.37 | Salt stress (NaCl) | No phenotype changes | [19] |
| Tobacco *Nicotiana tabacum* | Cu/Zn SOD | Pisum sativum | 3 | High light; low temperature | 3-4 fold higher activity of ascorbate peroxidise, higher photosynthetic rate | [20] |
| Sugarbeet *Beta vulgaris* L. inbred line 028 | Cytosolic Cu/ZnSOD<br><br>Chloroplastic Cu/ZnSOD | *Solanum lycopersicum* | N.c. | Pure Cercosporin; inoculation by *Cercospora beticola* spores; methyl viologen | No phenotype changes | [21] |

*Continuetio of the table*

| Target plant | Gene | Gene resource | Total SOD activity increase*, up | Improved tolerance to stress, kind | Phenotype compared with control under favourable conditions | Ref. |
|---|---|---|---|---|---|---|
| *Cotton Gossypium hirsutum* cv. Zhongmiansuo 35 | GhCu/ZnSOD<br><br>GhCu/ZnSOD and GhAPX<br>GhCu/ZnSOD and GhCAT | Gossypium hirsutum | 1.6 (Chl – 4.4)<br>2.4 (Chl – 6.3)<br>2.6 (Chl – 7.7) | NaCl, methyl viologen | Higher vegetative growth rate and biomass production | [22] |
| *Rice Oryza sativa* var Pusa Basmati-1 | Cytosolic Cu/ZnSOD | Avicennia marina | 2 | NaCl, methyl viologen, drought | Not studied | [23] |
| *Rice Oryza sativa* var Pusa Basmati-1 | Cytosolic Cu/ZnSOD | Avicennia marina | 2 | NaCl, methyl viologen, drought | Not studied | [23] |
| *Populus tremula* x *Populus alba* | FeSOD | Arabidopsis thaliana | 10.4 | Methyl viologen, low $CO_2$ partial pressure | No phenotype changes | [24] |
| *N. tabacum* cv Petit Havana SR1 | FeSOD | Arabidopsis thaliana | 1.8 | Methyl viologen | Not studied | [25] |
| Maize *Zea mays* var H99 | FeSOD | Arabidopsis thaliana | N.c. | Methyl viologen | Higher vegetative growth rate and biomass production | [26] |
| Canola *Brassica napus*, cv Mariia | CYP11A1 gene encoding cytochrome $P450_{SCC}$ | Bovine adrenal cortex mithochondria | 1.76 | Osmotic stress (mannitol); heat (42єC) 16 hours in growth chamber | Higher biomass production, TSP content, vegetative growth rate, faster germination and flowering | [27–29] |
| Canola, cv Magnat | Alpha 2b interferon (HuInfб2b) | Human | 1.25 | Osmotic stress (mannitol) | Higher biomass production, higher chlorophyll and carotenoid content | [30] |
| Arabidopsis thaliana | *TsRfBP* (riboflavin-binding protein) | *Trionyx sinensis japonicas* (soft-shelled turtle) | 1.1 | Drought (PEG 6000) | Higher vegetative growth rate and biomass production | [31] |
| Tobacco *Nicotiana tabacum* | ICE1 | Vitis amurensis | 3 | Cold (-4 єC) | Lower chlorophyll content | [32] |
| Tobacco *Nicotiana tabacum* | *OxO* (oxalate oxidase) | Triticum aestivum | 1.3–1.35 | Methyl viologen, high light | Cu/ZnSOD, CAT1, chloroplast APX, cytosolic APX and GR transcripts were more abundant | [33] |

*Continuetio of the table*

| Target plant | Gene | Gene resource | Total SOD activity increase*, up | Improved tolerance to stress, kind | Phenotype compared with control under favourable conditions | Ref. |
|---|---|---|---|---|---|---|
| Tomato *Solanum lycopersicum* | *ZAT12* encoding a $C_2H_2$ zinc finger transcription factor | Brassica carinata | 1.69 | Drought | No phenotype changes | [34] |
| Rice *Oryza sativa* L. ssp. japonica cv. Zhonghua 11 | *OsSAMDC* (S-adenosyl methionine decarboxyl-ase | down regulation of own gene via RNA interference | Decrease up to 1.2-fold | Drought, salinity | Seed germination, plant length, pollen viability, seed setting rate, grain yield per plant, and net photosynthetic rate were lowered | [35] |
| Medicago truncatula | *MtTdp2a* (ty-rosyl-DNA phosphodies-te-rase 2) | Medicago truncatula | N.c. (*SOD* transcripts were up to 1.4-fold) | Methyl viologen, ciprofloxacin, osmotic stress (PEG 6000) | Higher biomass production, transcripts of APX were more abundant | [36] |

N o t e s: * – calculated on the basis of protein content; N.c. – not calculated; Chl – chloroplastic, Ref. – references.

[10, 13, 23, 25]). But often they produce larger biomass [11, 15, 17–18, 22, 26–27, 29–31, 36], have shorter vegetative stage of development [11, 22, 26, 29, 31], were characterized by higher total soluble protein content [27–29] and changes in photosynthetic apparatus [20, 30, 32, 35] in comparison with wild type ones and/or transgenic plants bearing only empty vector. For most of them the improved tolerance to stresses of different origin has been proved in laboratory and field trials. So, biotechnological plants characterizing by increased SOD activity in non-stressed conditions may have higher adaptive capacity compared ones possessing lowered SOD activity.

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## ALTERATIONS IN SUPEROXIDE DISMUTASE ACTIVITY DUE TO HETEROLOGOUS GENE EXPRESSION: PLANT PECULIARITIES

***Aims.*** The analysis of the information about the features of transgenic plants bearing some genes which influenced on the activity of one from enzymes of plant antioxidant defence, namely superoxide dismutase, was the aim. ***Results.*** The review presents and summarizes the literature and own data reflecting physiological, biochemical and molecular biological parameters of plants possessing higher or lower superoxide dismutase activity due to transgenesis. ***Conclusions.*** Transgenic plants having the increased SOD activity would demonstrate no phenotype changes under favourable conditions (or these changes have not been studied). But often they produced larger biomass and were characterized by higher vegetative growth rate, higher total soluble protein and changed chlorophyll content in comparison with wild type ones and/or transgenic plants bearing only empty vector. For most of them the improved tolerance to stresses of different origin has been proved in laboratory and field trials. Biotechnological plants characterizing by increased SOD activity in non-stressed conditions may have higher adaptive capacity compared ones possessing lowered SOD activity.

*Keywords:* SOD activity, transgenic plants, favourable conditions, stress.