## Subcellular compartmentalization of the plant antioxidant system: An integrated overview

This is a very interesting review which summarizes much of the scientific knowledge about antioxidants systems in plants, putting together aspects of biochemistry, plants biology and subcellular integration.

The review is correctly organized in different sections including different fields of the Antioxidant System (components, regulation, compartmentalization, modelling). It is appreciable that a large bibliographic research was done, including both up-to-date and also some outdated bibliography

Some considerations to address:

#### IN GENERAL:

English grammar requires extensive editing. Authors should pay careful attention to some phrases or sentences which are confusing.

#### **INTRODUCTION**

Line 60: "...by lipid oxidizing" would be "...by lipid oxidation"?

Line 63: the death

Line 69: as shown in figure 2, once oxidative stress does happen, AOS protects the cells (or tissues) against oxidative damage.

Line 70: why would the authors say it is "excessive"? Do they mean there are more copies than necessary? Please rephrase this sentence.

Line 73: ...about 40 genes encodes antioxidant enzymes.

Line 77: when authors say "...which represent...", maybe they wanted to say "represented by"?

Line 81: I would say "by the mechanism summarized in Fig 1"

Line 85: I do not understand what they mean by "disproportionate".

I think more up-to-date examples have to be used. The authors mention obsolete techniques such as northern blot analysis to measure transcription levels, and there is abundant bibliography of antioxidant enzymes genes expression levels measured by qPCR (Kong *et al.*, 2014; Petriccione *et al.*, 2015; Amalia and Widiyanto, 2016; El-argawy and Adss, 2016)

Line 91: "The antioxidant activity of..."

Line 92: "also revealed that the activity <u>is</u> highly correlated with the expression level of mRNA". It would be correct to say: ...gene expression, or transcript or mRNA levels/abundance, etc.

Line 92 bis: Reference missing (Tiedge et al, 1997)

Line 96: same as Line 92

Authors mention many examples of activities and expression of different enzymes. However, they are not saying in which species they take place, missing the diversity of biological responses.

Line 98: "... (in pea) ..." could be written between commas. Just a suggestion.

Line 103: "...investigation of Alscher et el..." should be "...an investigation of Alscher et el..."

Line 124: "some facts about the regulation of this system during the stress" should be "...during stress".

#### SURVEY METHODOLOGY

In my opinion, an impersonal form should be used, without so many "we".

#### MAIN FEATURES OF THE ANTIOXIDANT SYSTEM COMPONENTS

Line 141: I find it hard to understand the objective of the whole sentence.

Line 152: In the paper of Maiorino et al 1988, there is no mention of APX activity and the GPX they work with is not mentioned to come from plants.

Line 161: Please, use the formal "it is" instead of the informal form "it's"

Line 168: "founded" should be "found"

Line 169: "...and showed that saturation of enzyme by hydrogen peroxide." The predicate of the sentence is missing or the intention of this sentence is not clear.

Line 173: "Kinetics of superoxide dismutase, SOD, EC 1.15.1.1, reaction can be described as first-order (Sawada and Yamazaki, 1973)" could be "Kinetics of superoxide dismutase (SOD, EC 1.15.1.1) reaction can be described as first-order (Sawada 174 and Yamazaki, 1973)".

Line 184: same as Line 161

Line 188: "Ascorbate present..." should be "Ascorbate is present..."

Lines 189-191: "It was found that adding exogenous ascorbate is increased regeneration processes and induce the development of fruiting bodies in *Hypsizygus marmoreus* by Chen et al. (2020).". Maybe you meant "that adding exogenous ascorbate increases the regeneration process and induces the development of fruiting bodies...".

"...by Chen et al (2020)" could be at the beginning: "Chen et al (2020) found that..."

Even when ascorbate is present in most life forms, in my opinion, it should be clear that the example the authors mention occurs in one species of fungi.

Lines 194-196: Do the authors mean "remediation of glutathione" or "remediation by/with glutathione"? It changes the meaning of the sentence.

Line 202-203: "...(with using thioredoxins to recharge themselves)...". It is not clear the meaning of this sentence. By "recharge" do you mean "reduce", "regenerate" or do you mean something else

EXPERIMENTAL DATA CONCERNING THE ANTIOXIDANT SYSTEM COMPONENTS

Line 210: "Some studies for tomato show tissue- organelle and stress-dependent differences...". Authors should keep the same verb tense.

Line 213: "Investigation of Mittova...". Authors should use "An investigation..." or "Investigations..."

Line 215: "...two contrast tomato species..." should be "...two different contrasting tomato species...".

Use commas to indicate which species is wild, salt-tolerant and which one is cultivated.

Line 216: "...the authors paid attention to..." instead of "...paid attention that..."

Line 217: "...is regenerated mainly by..."

Line 218: "... a higher SOD/APX ratio...corresponded to..."

Line 220: "A study of ROS...showed..."

Line 221: "(mainly superoxide)"

Line 222: What is being expressed is the gene that corresponds to that enzyme, not the enzyme itself.

Line 224: "New methods of measurements of antioxidant capacity still find out, for example, ferric-bipyridine assay (Naji et al., 2020)". The meaning of this sentence is not clear.

Line 226: Even when "...the ROS..." is not incorrect, it is more likely to be found as just "ROS".

Line 227: Who are the authors referring to when they say "we" in this phrase: "we don't have the ways to precisely count ROS in compartments yet"?

Do they mean they couldn't measure subcellular ROS with TEM or with any other method?

Line 238: "The study of AOS components and glyoxalase systems of different genotypes of wheat cultivars used for detecting their resistance". It is not clear the use of the word "used".

Line 244: "...This study also showed that exogenously applied sulfur helps for protection against salt stress by enhancing the AOS...". How do they do so? Which component of the AOS does sulfur enhance? I find it important since in the latter sentence, the authors made this difference when talking about salt stress.

Line 261: Hydrogen peroxide generation ratios units are expressed as nmol m<sup>-2</sup>s<sup>-1</sup>. Authors should mention what m<sup>-2</sup> stands for (leaf surface, in this case).

Line 273-276: please check the grammar in order to make the sentence clear.

# STRESS-RESPONSE OF THE ANTIOXIDANT SYSTEM COMPONENTS

Line 310: when mentioning the name of a species for the first time, the complete binomial name should be written.

# SUBCELLULAR COMPARTMENTALIZATION OF THE ANTIOXIDANT SYSTEM COMPONENTS

Line 378: "...oxygen, which is produced in high light conditions...", the same phrase was written in the previous sentence.

Lines 378-380: Please rewrite this sentence to get a clear idea.

### MODELING APPROACHES TO STUDY THE ANTIOXIDANT SYSTEM

Line 465: "...GR, APX and superoxide dismutase..." should be "...GR, APX and SOD..."

# **CONCLUSION**

Line 527: "...and would drug the experimental research towards new horizons." Maybe the authors meant "...and would drag the experimental research towards new horizons".

Amalia, L. and Widiyanto, S. N. B. (2016) 'Catalase ( CAT ) and Ascorbate Peroxidase ( APX ) Genes Expression Level in Growth of Banana Plantlets ( Musa acuminata ) cv . Ambon Lumut Under Chromium Stress Condition', *Journal of Plant Sciences*. Science Alert, 11(4), pp. 69–74. doi: 10.3923/jps.2016.69.74.

El-argawy, E. and Adss, I. A. (2016) 'Quantitative Gene Expression of Peroxidase , Polyphenoloxidase and Catalase as Molecular Markers for Resistance against Ralstonia solanacearum', (April), pp. 88–100.

Kong, Q. et al. (2014) 'Screening Suitable Reference Genes for Normalization in Reverse Transcription Quantitative Real-Time PCR Analysis in Melon', 9(1), pp. 1–11. doi: 10.1371/journal.pone.0087197.

Petriccione, M. *et al.* (2015) 'Reference gene selection for normalization of RT-qPCR gene expression data from Actinidia deliciosa leaves infected with Pseudomonas syringae pv .', *Nature Publishing Group.* Nature Publishing Group, (August), pp. 1–12. doi: 10.1038/srep16961.