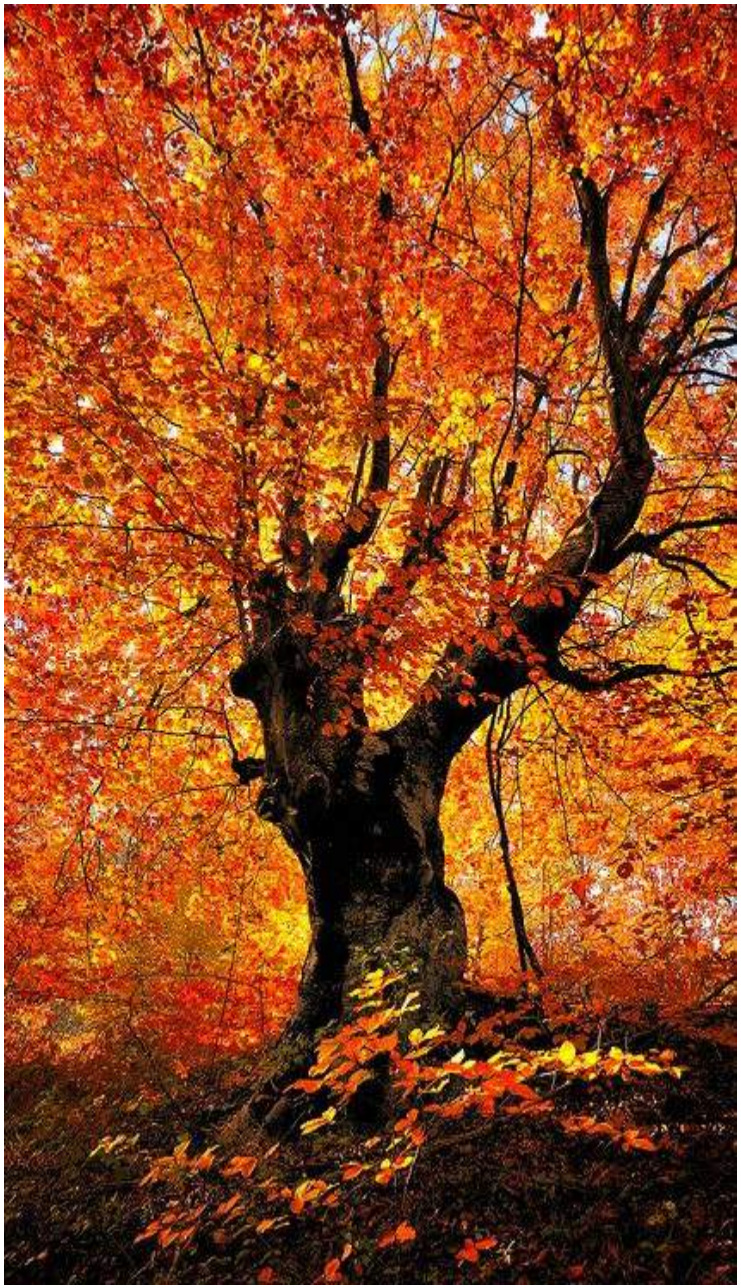
Antioxidant activity of European beech from different locations in Serbia

Dubravka Štajner[1], Boris M. Popović[1],
Saša Orlović[2], Ružica Ždero-Pavlović[1],
Marko Kebert[2], Vladislava Galović[2] i Branislav Trudić[2]

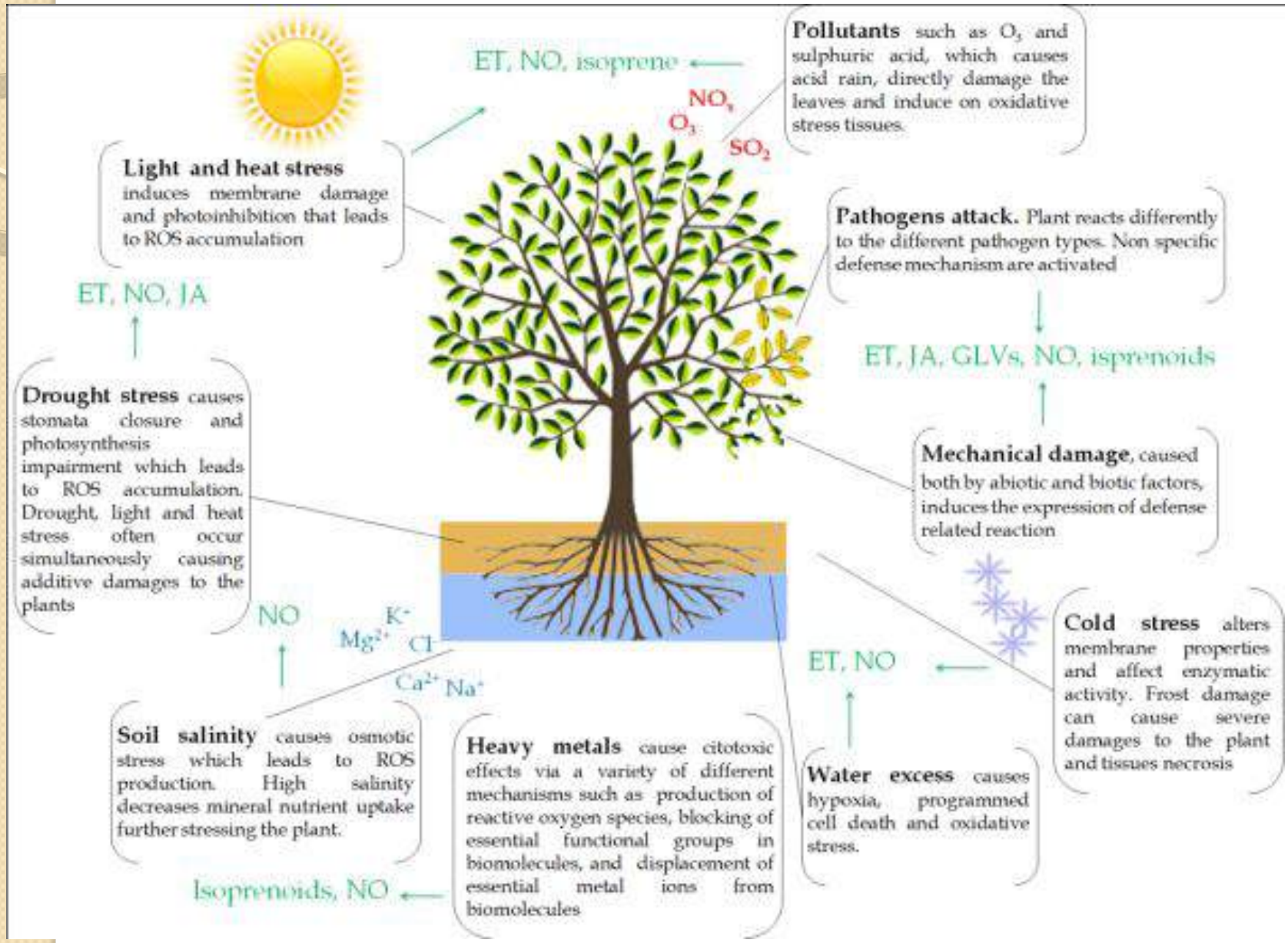
[1] *Faculty of Agriculture in Novi Sad*
[2] *Institute of Lowlend Forestry and Environment in Novi Sad*

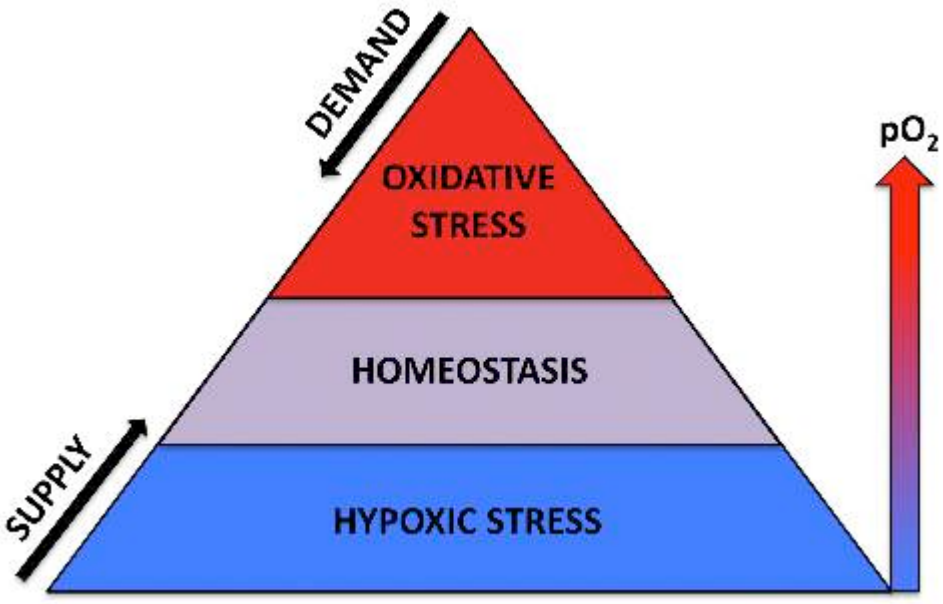


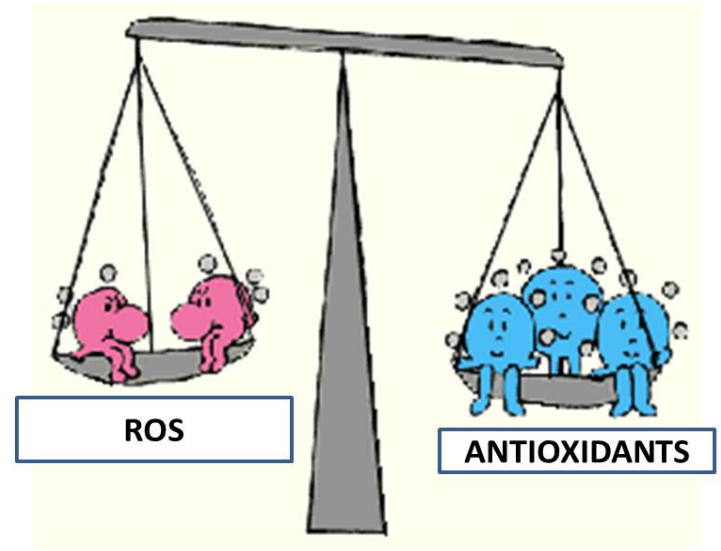
- European beech (*Fagus sylvatica* L.) is the most abundant tree species in Serbia and is highly interesting for both economic and ecological reasons.



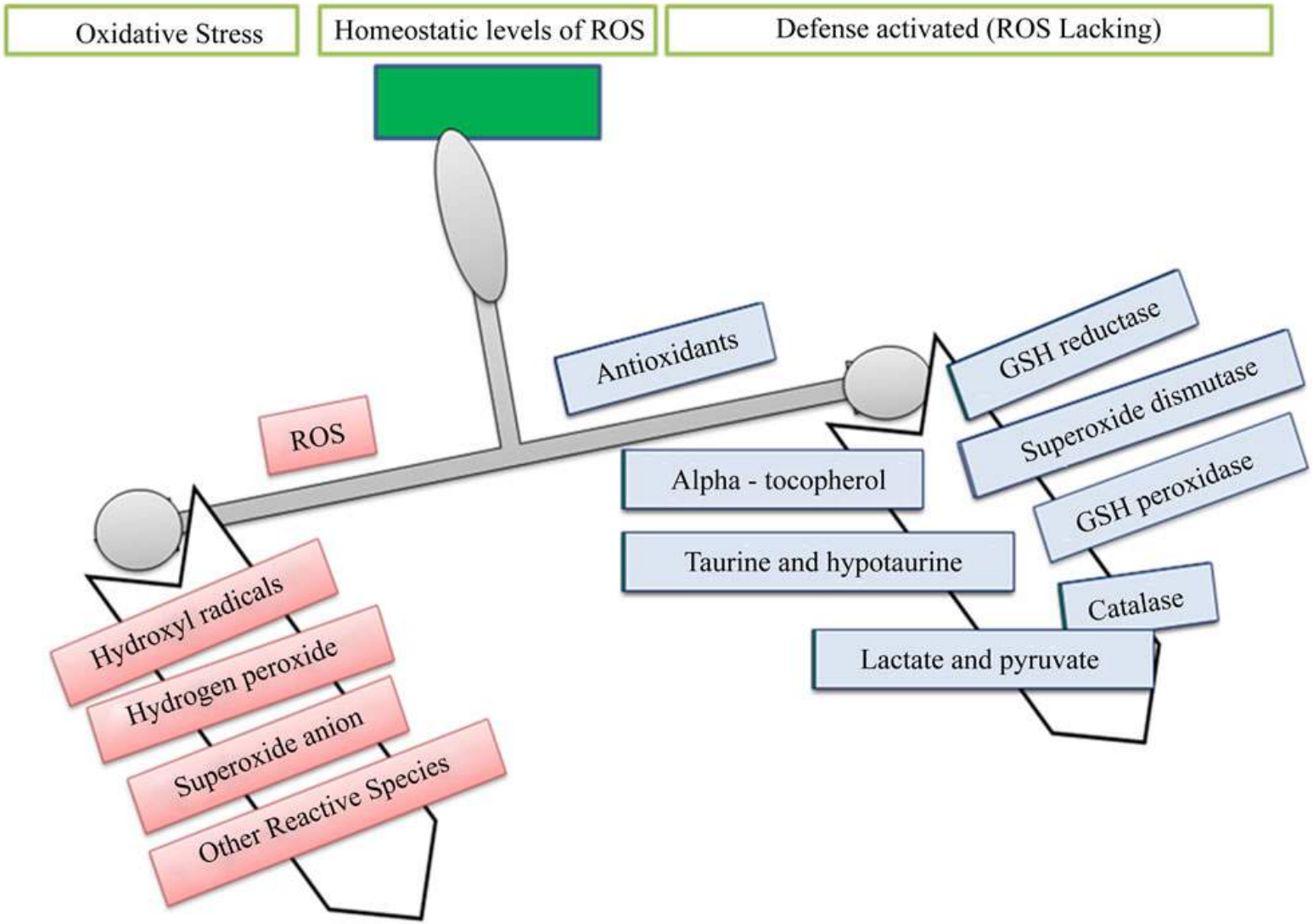
- It is well known that beech is a highly drought sensitive and its likely to suffer from climatic conditions prognosticated for the current century.


ET, NO, isoprene
Pollutants such as O_3 and sulphuric acid, which causes acid rain, directly damage the leaves and induce on oxidative stress tissues.
NO_x
O_3
SO_2
Light and heat stress induces membrane damage and photoinhibition that leads to ROS accumulation
Pathogens attack. Plant reacts differently to the different pathogen types. Non specific defense mechanism are activated
ET, NO, JA
ET, JA, GLVs, NO, isprenoids
Drought stress causes stomata closure and photosynthesis impairment which leads to ROS accumulation. Drought, light and heat stress often occur simultaneously causing additive damages to the plants
Mechanical damage, caused both by abiotic and biotic factors, induces the expression of defense related reaction
NO
K^+
Mg^{2+}
Cl^-
Ca^{2+}
Na^+
ET, NO
Cold stress alters membrane properties and affect enzymatic activity. Frost damage can cause severe damages to the plant and tissues necrosis
Soil salinity causes osmotic stress which leads to ROS production. High salinity decreases mineral nutrient uptake further stressing the plant.
Heavy metals cause citotoxic effects via a variety of different mechanisms such as production of reactive oxygen species, blocking of essential functional groups in biomolecules, and displacement of essential metal ions from biomolecules
Water excess causes hypoxia, programmed cell death and oxidative stress.
Isoprenoids, NO





DISEASE ⟷ HEALTH


Oxidative Stress
Homeostatic levels of ROS
Defense activated (ROS Lacking)
ROS
Antioxidants
Hydroxyl radicals
Hydrogen peroxide
Superoxide anion
Other Reactive Species
Alpha - tocopherol
Taurine and hypotaurine
Lactate and pyruvate
GSH reductase
Superoxide dismutase
GSH peroxidase
Catalase

Previous works:

- **Popović, B.M.,** Štajner, D., Ždero, R., Orlović, S., Galić Z. Antioxidant characterization of oak extracts combining spectrophotometric assays and chemometrics, *The Scientific World Journal*, (2013) Article ID 134656, 8 pages.

- Štajner, D., **Popović, B.M.,** Ćalić, D., Štajner M. Comparative study of antioxidant status in androgenic embryos of *Aesculus hippocastanum* and *Aesculus flava*, *The Scientific World Journal*, (2013) Article ID 767392, 7 pages.

- Štajner, D., Orlović, S., **Popović, B.M.,** Kebert, M., Stojnić, S., Klašnja B., Chemical Parameters of Oxidative Stress Adaptability in Beech, *Journal of Chemistry* (2013), 8 pages.

- Trudić, B., Kebert, M., **Popović, B.M.,** Štajner, D., Orlović, S., Galović V., The level of oxidative stress in Poplars due to heavy metal pollution in soil, *Baltic Forestry*, 18 (2) (2013) 214-227.

- Trudić, B., Kebert, M., **Popović, B.M.,** Štajner, D., Orlović, S., Galović, V., Pilipović A. The effect of heavy metal pollution in soil on Serbian Poplar clones, *Šumarski list*, 5–6 (2013) 287–296.

- Štajner D., Orlović S., **Popović B.M.**, Kebert M, Galić Z. Screening of drought oxidative stress tolerance in Serbian melliferous plant species, *African Journal of Biotechnology* 10 (9), (2011), 1609-1614.

Objective of the investigation:

- The present study was designed to define and compare antioxidant activity of leaf extracts of *Fagus sylvatica* L. from three different locations in Serbia (Tara, Kopaonik and Stara planina). Parameters of oxidative stress and pigment contents were also investigated.

Parameters

Oxidative stress: lipid peroxidation (LP)

Drought stress: proline (Pro)

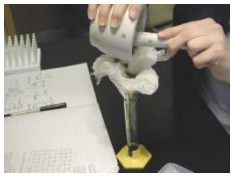
Phenolic parameters (total phenols, flavonoids, tannins and proantocyanidines)

Antioxidant activity (FRAP, PRAC, DPPH, OH, NO, O_2^- RSC, ESR)

Pigments (Chla, Chlb, Car)

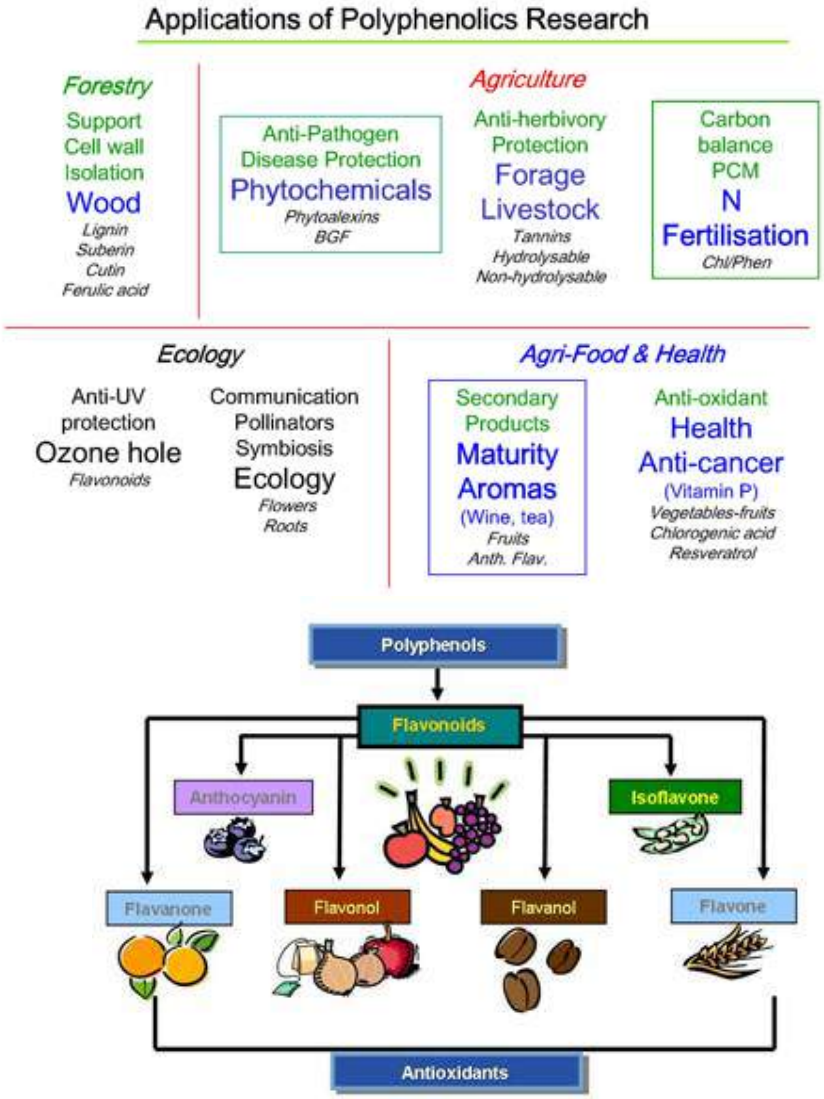
Extraction procedure for LP with TCA, for Pro with toluene and for pigments with acetone

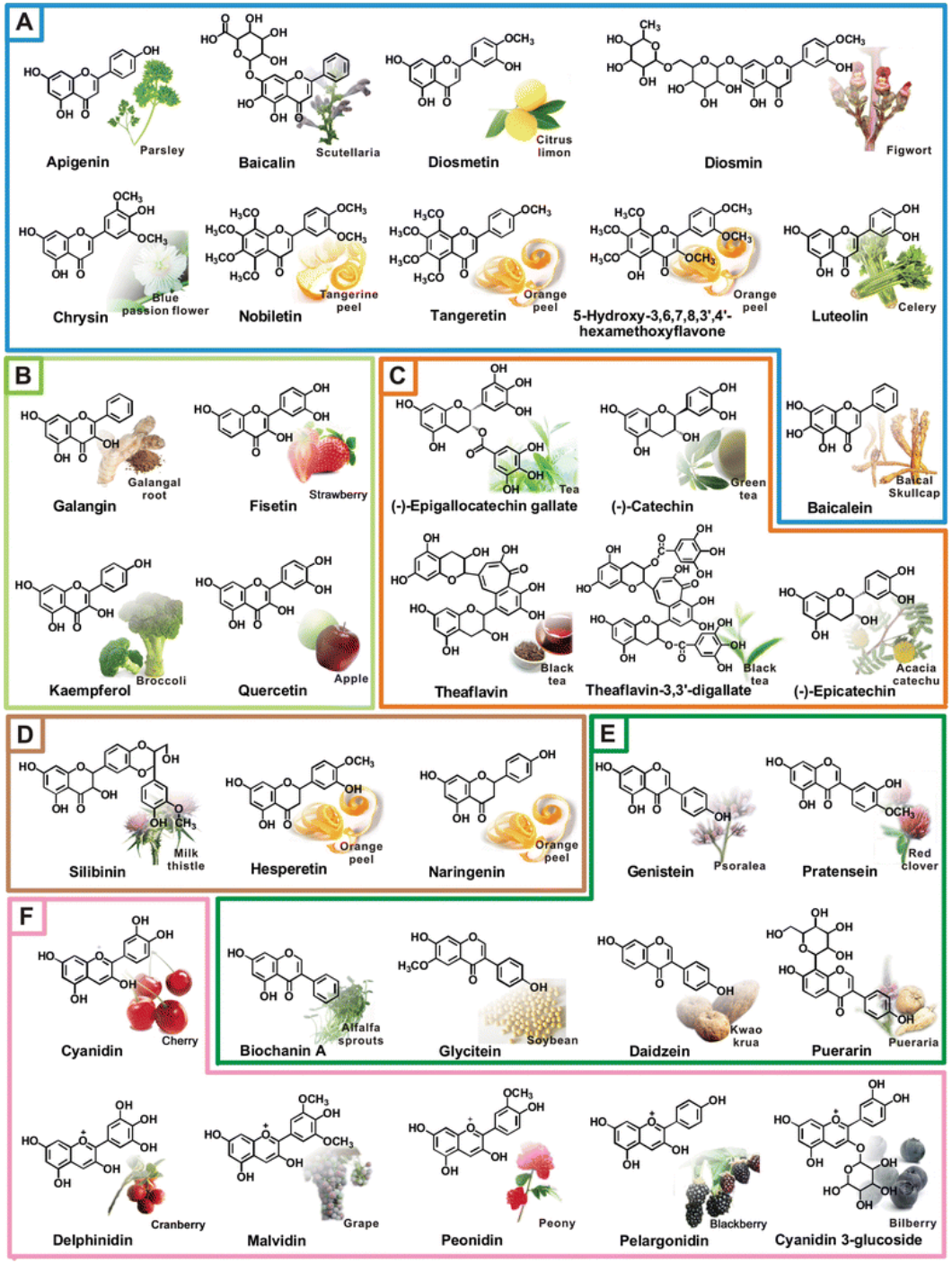


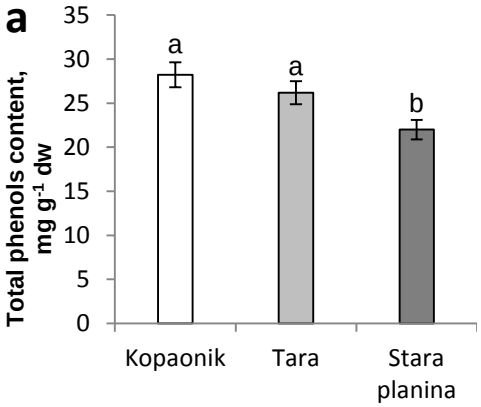

Phenolic parameters and antioxidant activity were analysed from ethanolic extracts

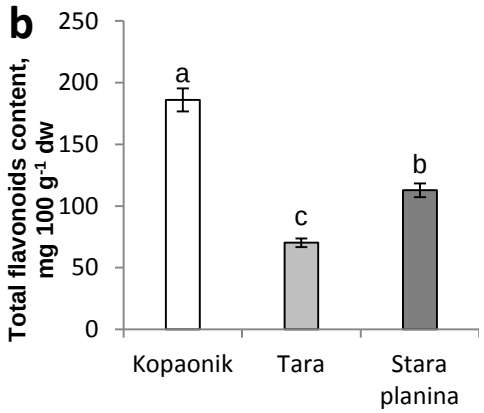
Polyphenolic parameters

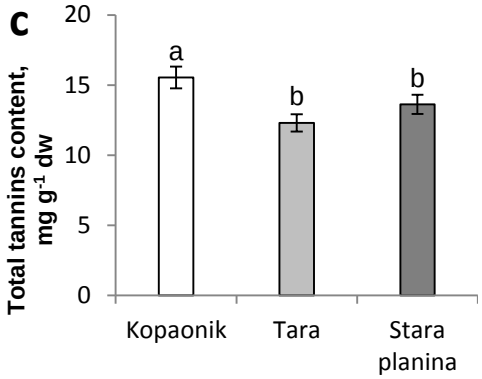


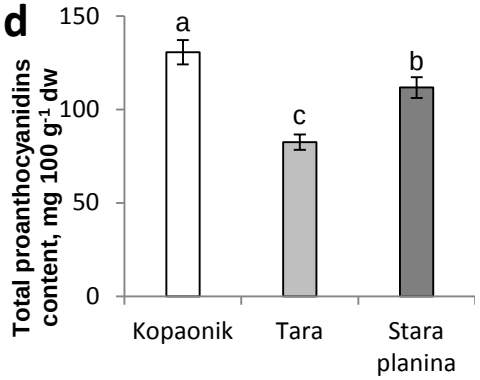

A
Apigenin
Parsley
Baicalin
Scutellaria
Diosmetin
Citrus limon
Diosmin
Figwort
Chrysin
Blue passion flower
Nobiletin
Tangerine peel
Tangeretin
Orange peel
5-Hydroxy-3,6,7,8,3',4'-hexamethoxyflavone
Orange peel
Luteolin
Celery
B
Galangin
Galangal root
Fisetin
Strawberry
Kaempferol
Broccoli
Quercetin
Apple
C
(-)-Epigallocatechin gallate
Tea
(-)-Catechin
Green tea
Baicalein
Baical Skullcap
Theaflavin
Black tea
Theaflavin-3,3'-digallate
Black tea
(-)-Epicatechin
Acacia catechu
D
Silibinin
Milk thistle
Hesperetin
Orange peel
Naringenin
Orange peel
E
Genistein
Psoralea
Pratensein
Red clover
F
Cyanidin
Cherry
Biochanin A
Alfalfa sprouts
Glycitein
Soybean
Daidzein
Kwao krua
Puerarin
Pueraria
Delphinidin
Cranberry
Malvidin
Grape
Peonidin
Peony
Pelargonidin
Blackberry
Cyanidin 3-glucoside
Bilberry

Contents of total phenols (**a**), flavonoids (**b**), tannins (**c**) and proanthocyanidins (**d**)



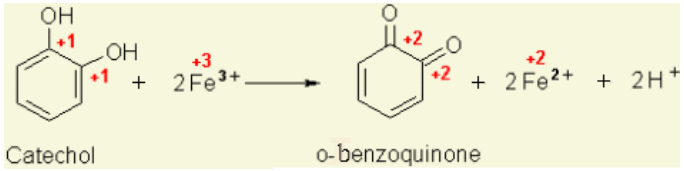


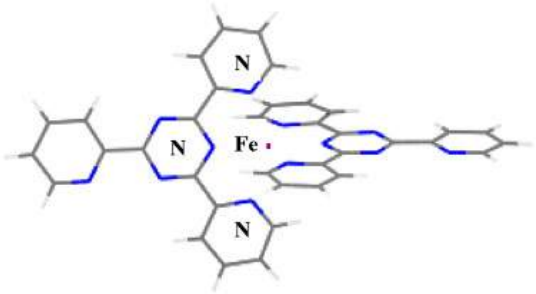




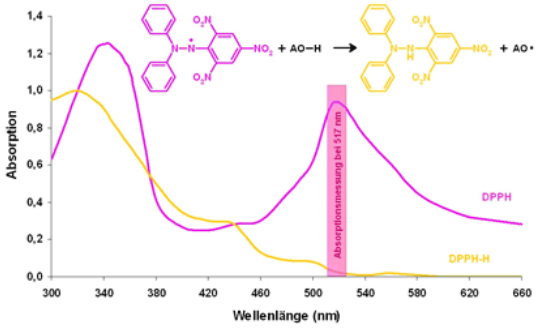
Antioxidant activity determination

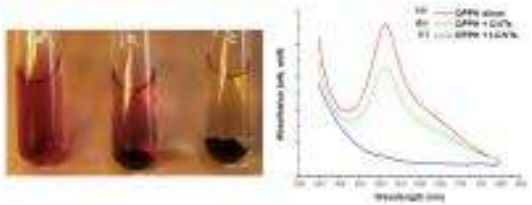
- **FRAP** method (Benzie&Strain, 1999.)

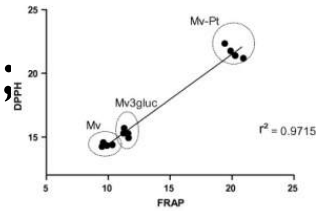


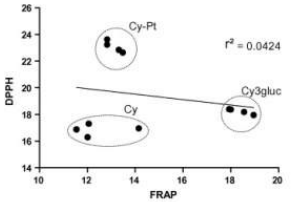


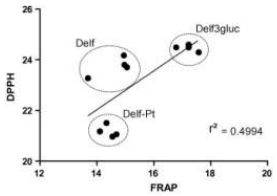
- **DPPH** radical scavenging capacity (Blois, 1958; Williams et al. (1995)



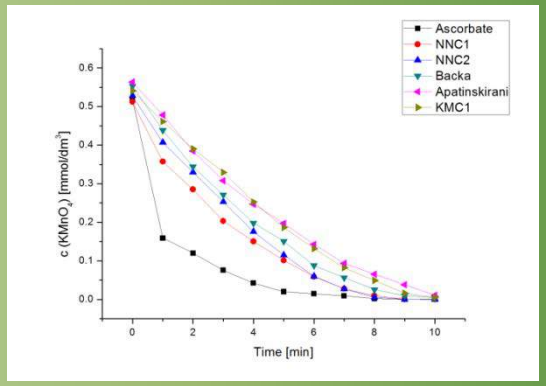








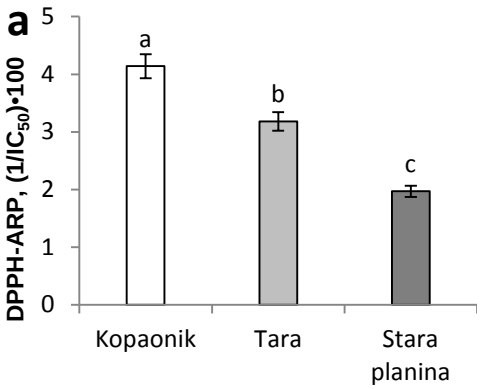
PRAC method

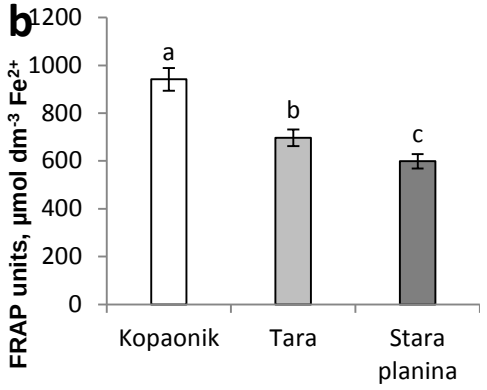


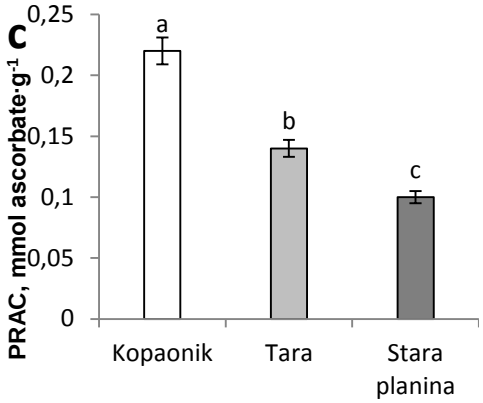
$$A_{50} = \frac{t_{(standard)}}{t_{(plant\ sample)}} \cdot \frac{c_{(standard)}}{m_{(plant)}} \cdot \frac{v_{(standard)}}{v_{(plant\ sample)}} \cdot V_{(extract)}$$

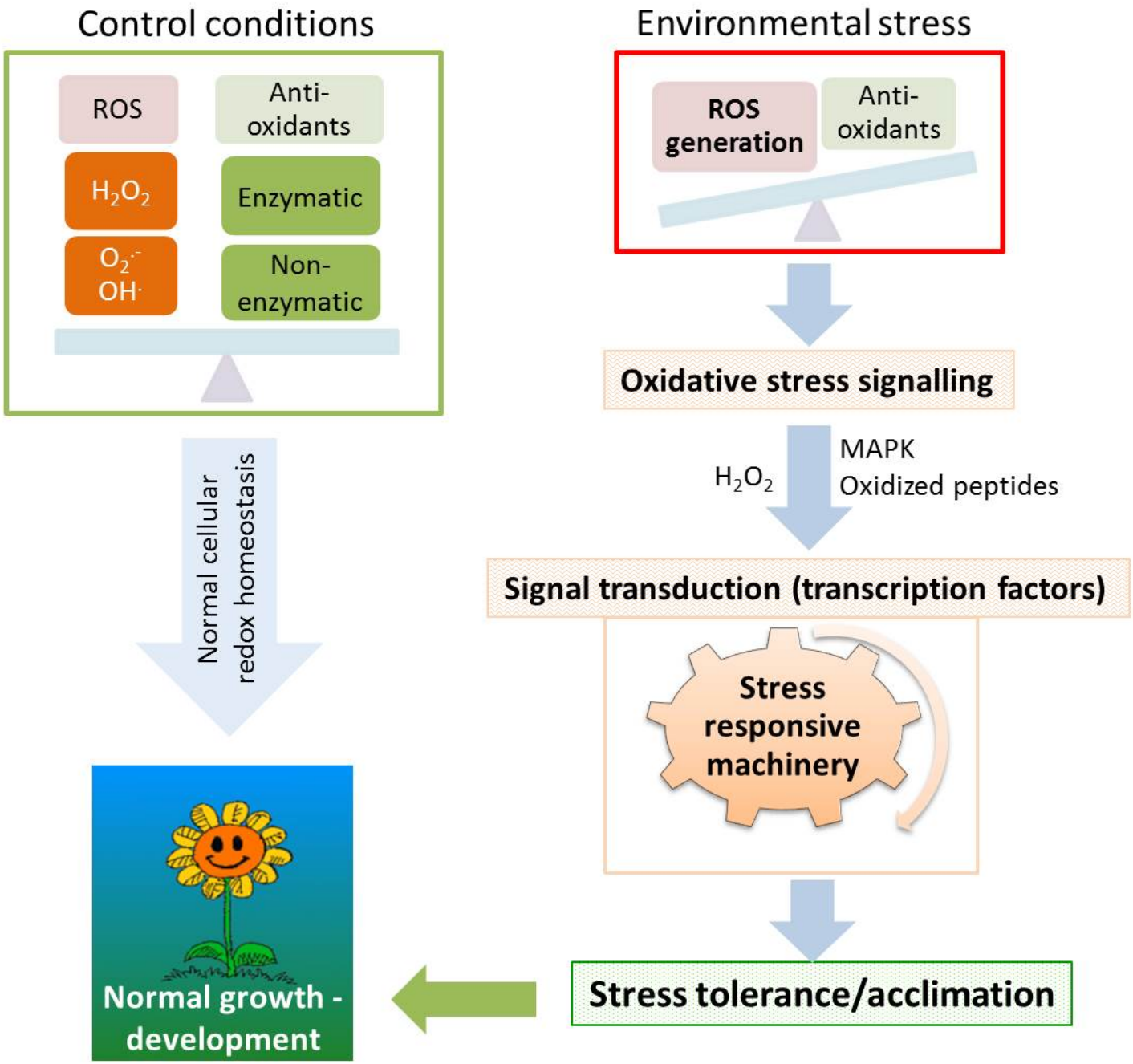
- Cacig et al., 2006, In: Zbornik Matice srpske za prirodne nauke.
-Popović et al, 2012, Food chemistry.

Scavenging activities of extracts against DPPH· (**a**), FRAP-ferric reducing antioxidant power (**b**) PRAC-permanganate reducing antioxidant capacity (**c**)

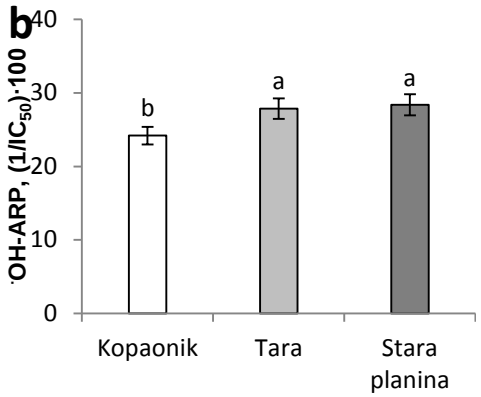


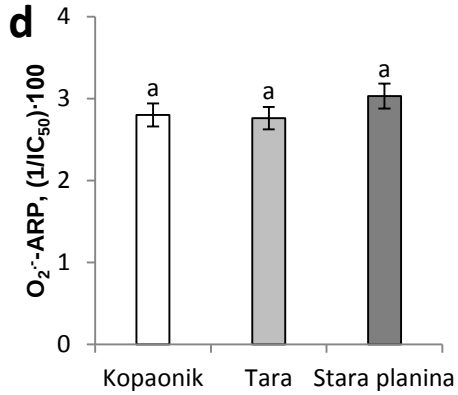


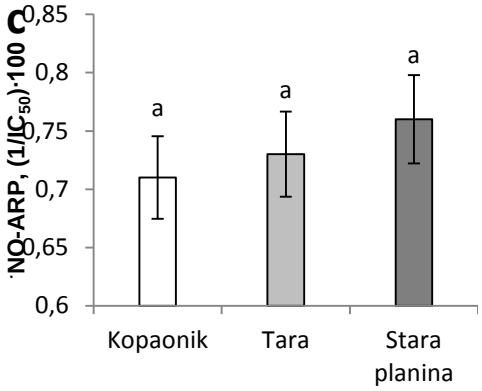



Control conditions
ROS
Anti-oxidants
H_2O_2
Enzymatic
$O_2^{\cdot-}$
$OH^{\cdot}$
Non-enzymatic
Environmental stress
ROS generation
Anti-oxidants
Oxidative stress signalling
H_2O_2
MAPK
Oxidized peptides
Signal transduction (transcription factors)
Stress responsive machinery
Stress tolerance/acclimation
Normal cellular redox homeostasis
Normal growth - development

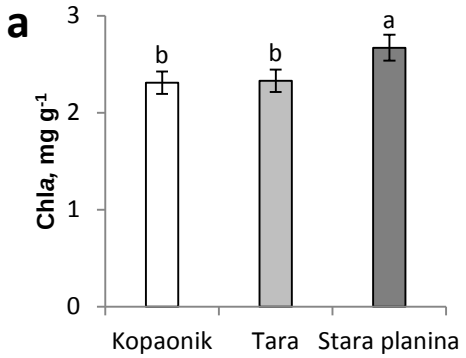
$^{\bullet}OH$ (**b**), $^{\bullet}NO$ (**c**), and $O_2^{\bullet-}$(**d**) antiradical power (ARP)

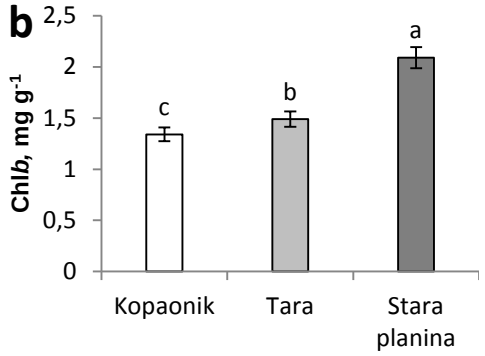


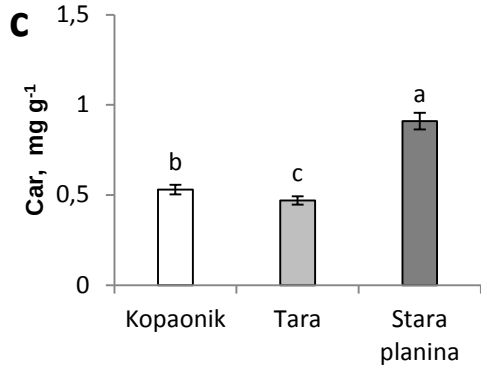




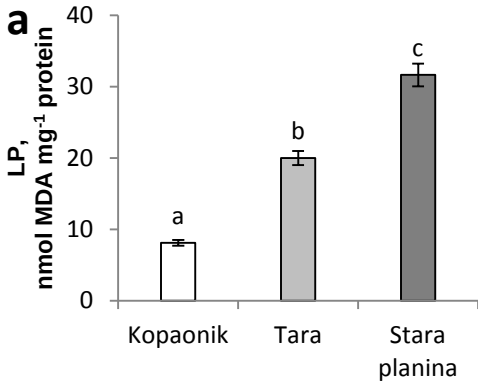
Contents of chlorophyll *a* (**a**), chlorophyll *b* (**b**) and carotenoids (**c**)

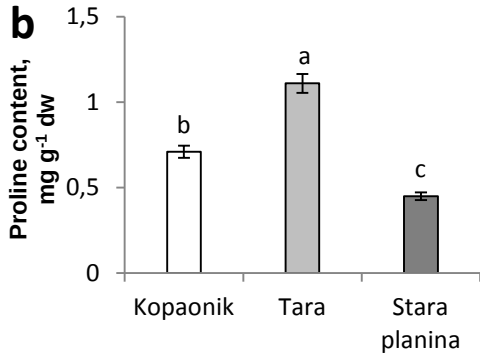


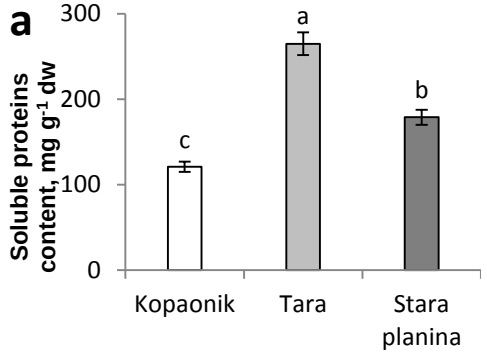




Lipid peroxidation, contents of soluble proteins (**a**), and proline (**b**)







Drought stress

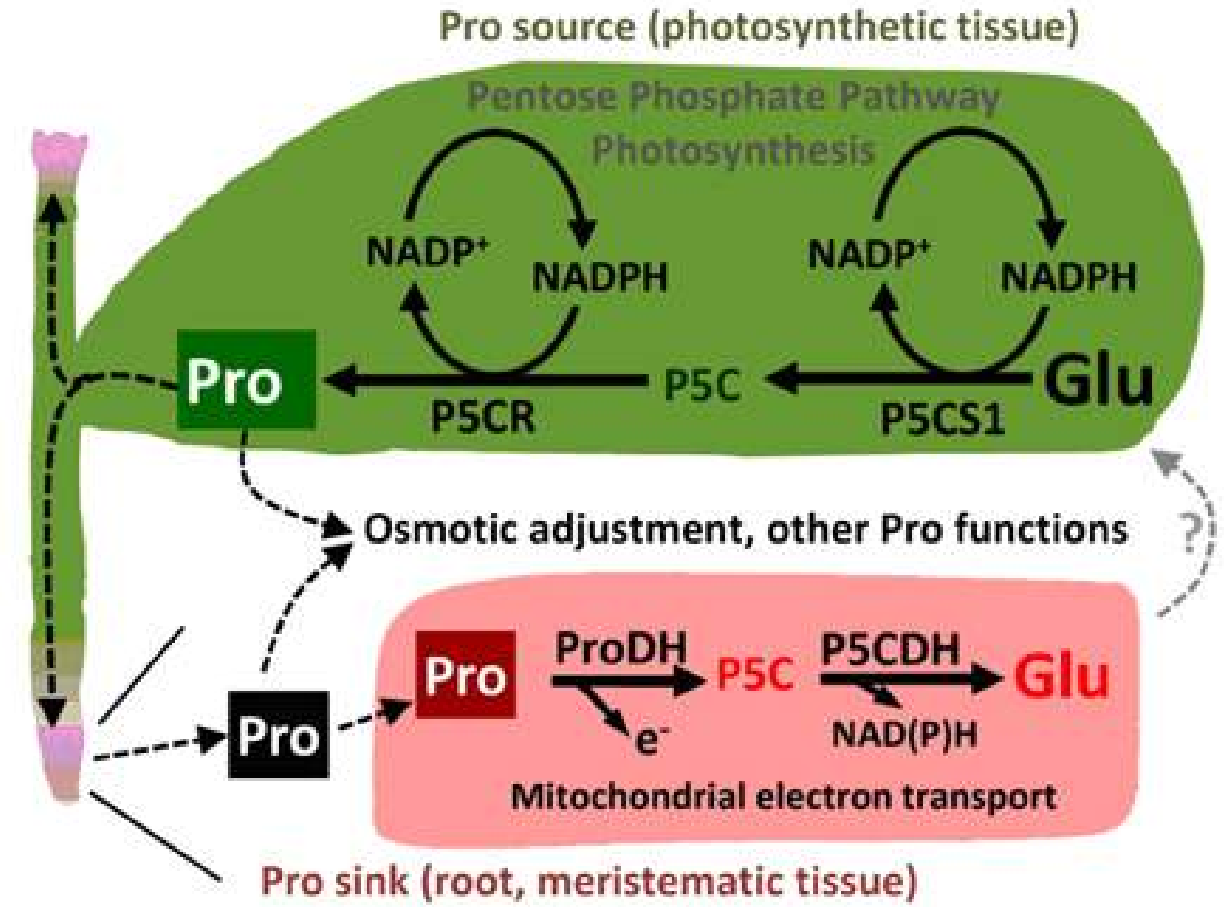


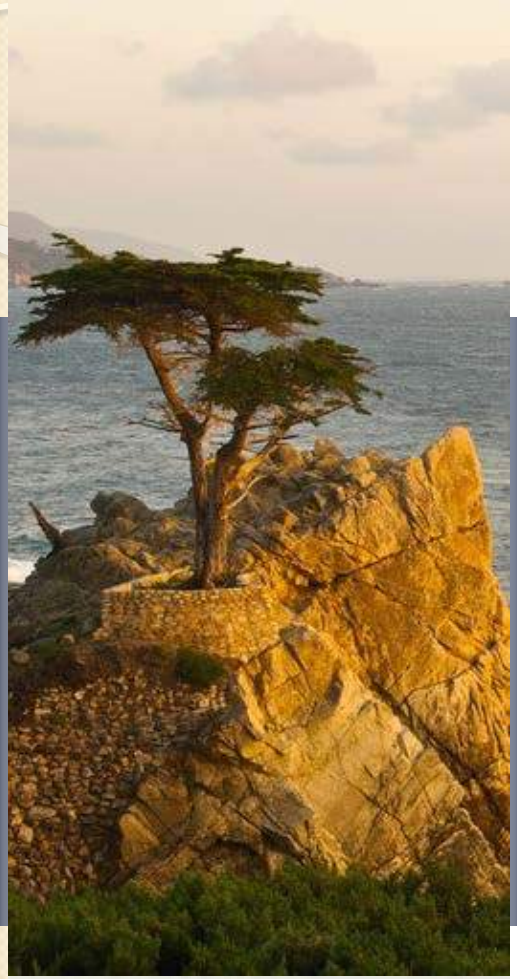
Figure 2. Essential role of tissue specific proline synthesis and catabolism in growth and redox balance at low water potential. From Sharma, Villamor and Verslues (2011) *Plant Physiology*

Conclusion

- Extract from Stara planina showed the highest LP, the lowest proline accumulation, lowest FRAP and PRAC values, the lowest DPPH antioxidant activity and the lowest polyphenol content. Results pointed out that beech population in Stara planina has been under negative influence of oxidative stress.

- The highest accumulation of proline and high accumulation of MDA were observed in beech extract from Tara what pointed out adaptive responses of this Beech population on drought stress.

- Extract from Kopaonik was characterized as containing the highest amount of phenols, flavonoids, tannins and proanthocyanidins. Also, the lowest accumulation of MDA was observed in same extract, what indicated high oxidative stress tolerance.



Final conclusion

Chemical parameters such as LP, Pro and antioxidant activity are useful oxidative stress markers and markers for environmental stress.

We can recomand these parameters as diagnostic critera for estimation of plant health and adaptation on abiotic stress.

"If you cut down a forest, it doesn't matter how many sawmills you have if there are no more trees."

~ Susan George
(social scientist and activist)

Research team

Prof. Dr Dubravka Štajner

Prof. Dr Boris Popović

M.Sc. Ružica Ždero-Pavlović

Prof. Dr Saša Orlović

Dr. Vladislava Galović

M.Sc. Marko Kebert

M.Sc. Branislav Trudić

Thank you for your attention!