Function of antioxidant enzymes and metabolites during maturation of pea fruits

Manuel A. Matamoros[a], Jorge Loscos[a,b] and Manuel Becana[a]

[a]Departamento de Nutrición Vegetal, Estación Experimental de Aula Dei, CSIC, Apdo. 13034, 50080- Zaragoza Spain. manumat@eead.csic.es

[b] Present adress: Université de Lausanne, Bât. Biophore, Lausanne, Switzerland.

INTRODUCTION

Leguminous plants are crops of major economical value. They are also essential to sustainable agricultural systems because of their ability to establish nitrogen-fixing symbioses with soil bacteria, thus providing a biological alternative to chemical fertilization. Nitrogen fixation, photosynthesis, respiration and peroxisomal metabolism involve electron transfer reactions that give rise to reactive oxygen species (ROS). Antioxidants include enzymes and metabolites that modulate the concentrations of ROS, avoiding their potential cytotoxicity while allowing them to function as signal molecules (Fig. 1).

Extensive studies on the role of ROS and antioxidants in fruit development and ripening have been conducted on climacteric fruits. However, the role of antioxidants in the development, maturation and post-harvest storage of legume fruits is poorly defined. Here we present results on the implication of antioxidants and oxidative stress in the maturation and post-harvest storage of pea fruits. In addition, we assess the effect of sustainable agricultural practices on the antioxidant composition, nutritional value and post-harvest shelf life of pea fruits.

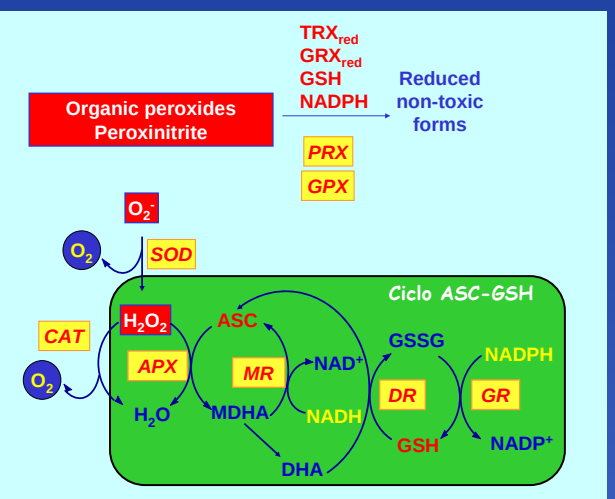


Fig. 1. Antioxidants in plants. SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; MR, monodehydroascorbate reductase; DR, dehydroascorbate reductase; GR, glutathione reductase; PRX, peroxiredoxin; GPX, glutathione peroxidase; ASC, reduced ascorbate; MDHA, monodehydroascorbate; DHA, dehydroascorbate; GSH, reduced glutathione; GSSG, oxidized glutathione; TRX, thioredoxin; GRX, glutaredoxin.

RESULTS

Antioxidant protection is similar in pea fruits from nodulated and non-nodulated plants. Fruits were separated into seeds and seedless pods immediately after harvest. In general, the antioxidant content was greater in the seeds, but there were also significant amounts in the seedless pods (Fig. 2). We conclude that the fruits from plants that are dependent on nitrogen fixation have similar antioxidant levels to those that are supplied with ammonium nitrate.

Peroxiredoxins are differentially expressed in pea tissues. Peroxiredoxins (Prx) play important roles in antioxidant defence and stress signalling. The chloroplastic isoforms (PrxQ, 2-CysPrx) are abundantly expressed in leaves, where they protect photosynthesis against ROS. 2-CysPrx was also detectable in pods. The cytosolic PrxIIc proteins were clearly present in seeds and pods, but were barely detectable in roots, nodules or leaves. The mitochondrial isoform PrxIIF was expressed in all five organs examined (Fig. 3).

Pea fruit maturation involves a decrease in antioxidant capacity but not oxidative stress. Ripening has been described as an oxidative process in climacteric fruits. Conceivably then, the antioxidant system may be involved in the control of fruit maturation. Antioxidants decreased during maturation of pea seeds, except SOD and DR activities, which increased. With few exceptions, antioxidant activities and metabolites followed similar trends in pods (Fig. 4). PrxIIc protein decreased in seeds but consistently accumulated in pods from overmature fruits. Despite the decrease in antioxidant defences, we could not detect oxidative stress using lipid and protein oxidation as markers during maturation. In addition, ascorbate and glutathione redox states remained unchanged. We surmise that the low levels of protein and lipid oxidation during the reproductive phase may be part of a strategy to limit the transfer of oxidatively damaged components to the offspring.

Prolonged storage of pea fruits decreases antioxidant capacity but does not cause oxidative damage. Storage of pea fruits at room temperature caused a general decrease of their antioxidant content, with the exceptions of SOD activity and GPX proteins (Fig. 5 A, B). In addition, we found a decrease of 60-70% in malondialdehyde and protein carbonyl groups, whereas the pools of ascorbate and glutathione remained >90% reduced. The more likely explanation for the absence of measurable oxidative damage or altered redox poise in cells is a general slow-down of metabolism during storage.

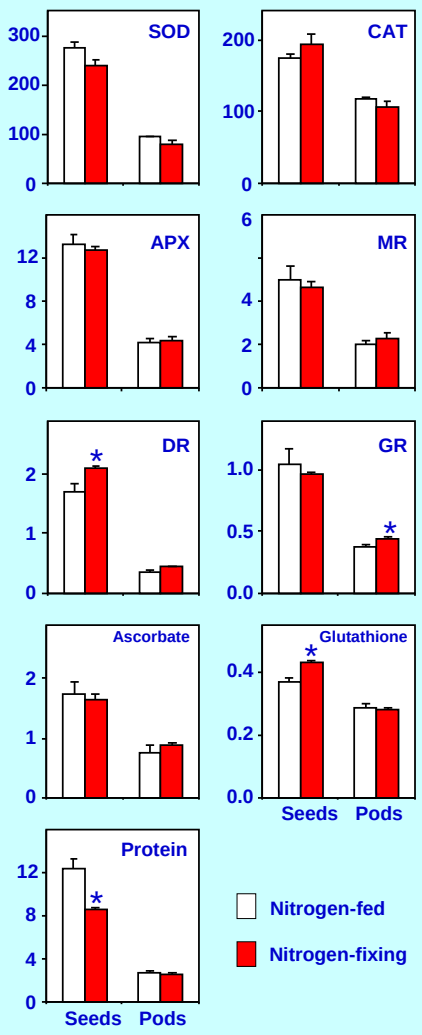
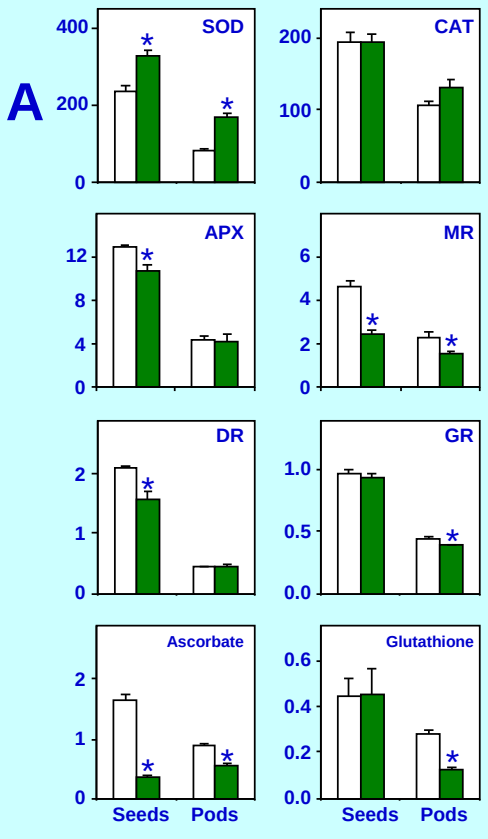


Fig. 2. Antioxidants in seeds and pods of nitrogen-fed and nitrogen-fixing pea plants. Enzyme activities are expressed in μmol min^{-1} g^{-1} fresh weight (FW), except SOD, which is expressed in units g^{-1} FW. Metabolites are expressed in μmol g^{-1} FW and protein in mg g^{-1} FW. Values are means ± SE of four to six replicates from at least three series of plants grown independently. For each tissue, means marked with an asterisk are significant at $P<0.05$ based on Student's *t*-test. *Abbreviations:* see Fig 1.



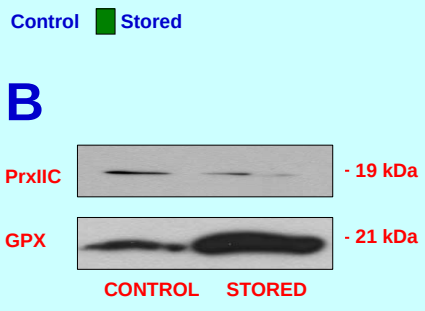


Fig. 5. Changes in antioxidants during storage of pea fruits. Control fruits were frozen in liquid nitrogen immediately after harvest, whereas stored fruits were kept at 25ºC for 4 d. (A) Enzyme activities and metabolite contents in seeds and seedless pods. Units and abbreviations are as described in Figs. 1 and 2. Values are means ± SE of four to six replicates from at least three series of plants grown independently. For each tissue, means marked with an asterisk are significantly different at $P<0.05$ based on Student's t-test. (B) Immunoblot analysis of cytosolic peroxiredoxin II (PrxIIc) and GPX in seeds. Gels were loaded with 30 μg (GPX) or 15 μg (PrxIIc) of protein per lane. Blots are representative of four gels loaded with seed extracts from different plants.

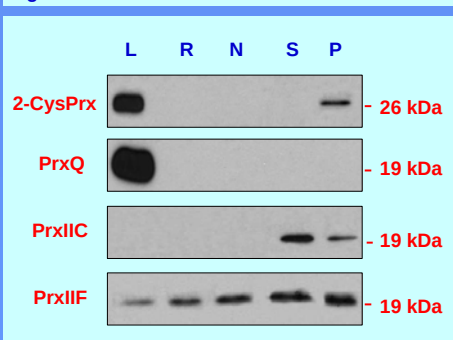


Fig. 3. Relative abundance of Prx proteins in leaves (L), roots (R), nodules (N), seeds (S) and seedless pods (P) of pea plants. Gels were loaded with 15 μg of protein per lane. Blots are representative of three to five gels loaded with extracts from different plants.

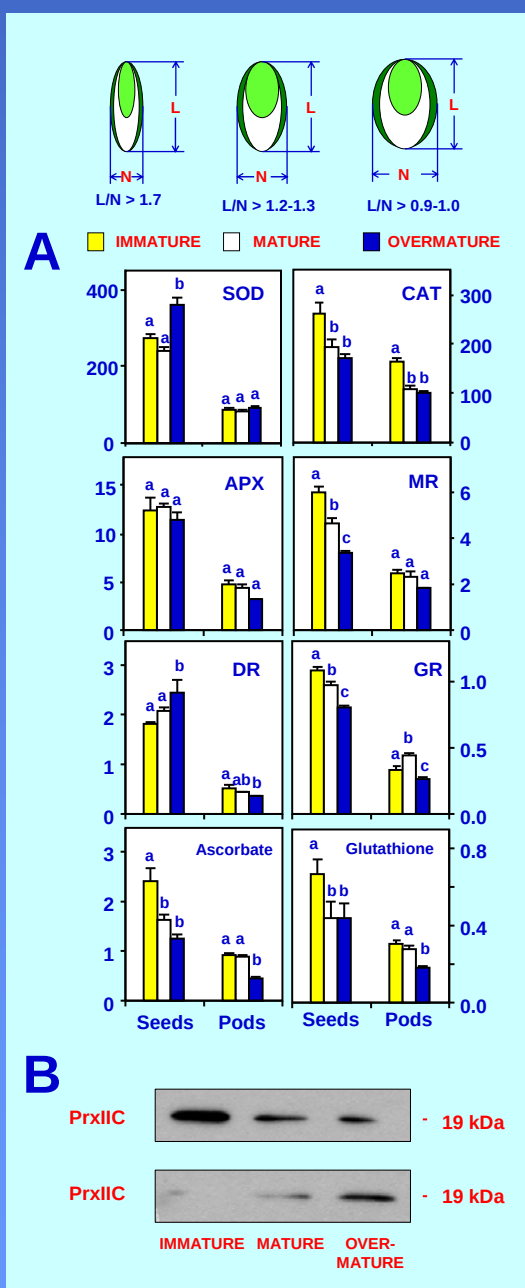


Fig. 4. Changes in antioxidants during maturation of pea fruits. These were classified in immature, mature or overmature according to their L/N ratio. (A) Enzyme activities and metabolite contents of seeds and seedless pods. Units and abbreviations are as described in Figs. 1 and 2. Values are means ± SE of four to six replicates from at least three series of plants grown independently. For each tissue, means denoted by the same letter do not significantly differ at $P< 0.05$ based on Duncan multiple range test. (B) Immunoblot analysis of cytosolic peroxiredoxin II (PrxIIc) in seeds and pods. Gels were loaded with 15 μg of protein per lane, and blots are representative of three to five gels loaded with extracts from different plants.

REFERENCES: Brennan & Frenkel (1977) *Plant Physiology* 59, 411-416; Jiménez *et al.* (2002) *Planta* 214, 751-758; Rogiers *et al.* (1998) *Annals of Botany* 81, 203-211.