The Quarterly Scientific Journal of Applied Biology

Vol.37, No.4, Sering.82, Winter 2024, p. 7-9

Journal homepage: https://jab.alzahra.ac.ir

doi.org/10.22051/jab.2024.45868.1603

Does Pretreatment with a Serine Protease Inhibitor Enhance Walnut Kernel Tolerance to Aging Condition?

Seyedeh Fatemeh Fallah[1], Farshid Ghaderi-Far[2], Masoud Golalipour[3], Hamid Reza Sadeghipour*[1]

[1]Department of Biology, Faculty of Sciences, Golestan University, Gorgan, Iran

[2]Department of Agronomy, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

[3]Department of Human Genetics, Gorgan University of Medical Sciences, Iran

***Corresponding author: h.r.sadeghipour@gmail.com**

Introduction

Seed aging refers to decline viability, vigor and quality over time due to storage. It is associated with changes in cell biochemical constituents (Kibinza et al., 2011). Proteins play an important role in seed vigor (Jiang et al., 2018). While alterations in lipids e.g., lipid mobilization during seed aging is well substantiated, little is known about changes in protein metabolism during this process. Kernels from Persian walnut contain up to 70 % oil and 17 % protein as reserve materials (Sze-Tao et al., 2000). It is known that the inhibition of kernel lipase activity increases their resistance to aging conditions (Pournik et al., 2019). However, the role of proteolytic processes in kernel aging has remained unknown. Therefore, we hypothesized that a) walnut kernel aging is associated with storage protein mobilization and, b) the inhibition of proteolytic activities can improve kernel viability under aging conditions. To test these hypotheses, seed germination as well as changes in some biochemical parameters related to protein metabolism were compared in walnut kernels pretreated with and without the serine protease inhibitor phenylmethylsulfonylfluoride (PMSF) enforced to aging by controlled deterioration (CD) treatment.

Material and Methods

Walnut kernels (*Juglans regia* L.) were soaked to moisture contents (MC) of 15 and 20 % with water (control) or phenylmethyl sulfonyl fluoride (PMSF, 1 mM) solution and then aged by

controlled deterioration (CD) after incubation at 45°C for 3 and 6 days. The experiments were carried out as factorial in a completely randomized design. Aged and non-aged Walnut kernels in different treatments were subsequently used for germination and biochemical studies. To assess germination, in each treatment, 30 walnut kernels (in three replicates each of 10), were surface sterilized (0.5 % sodium hypochlorite, 15 min), rinsed (3 times) with water, incubated in sand medium at 25°C with daily irrigation. The germination of walnut kernels was recorded up to 40 days. The kernel total amino acid, proline, total protein and soluble protein contents were determined according to Yemm and Cocking (1955), Bates et al. (1973), Markwell et al., (1981), and Bradford (1976), respectively. The level of protein carbonylation was determined according to Xia et al. (2016) using dinitro-phenyl hydrazine. Walnut kernels protease activity was qualitatively determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) zymography according to Heussen and Dowdle (1980). Detection of reduced disulfide bonds of proteins by Monobromobimane (mBBr) labeling and their detection by SDS-PAGE was carried out according to Kobrehel et al. (1992) and Fling and Gregerson (1986), respectively.

Result and Discussion

Controlled deterioration (CD) for 3 days reduced germination of kernels with 15 % MC, but no germination occurred for kernels with 20 % MC and after 6 days of CD there was no germination at any MC levels. Thus, aging compromised germination of kernels with 20 % MC more than 15 %. These are consistent with our previous study (Pournik et al., 2019), although in that study, walnut seeds with 15 % MC had decreased germination after 6 days aging. Likely, the effect of aging on seed germination depends on the variety and genotype.

Although total kernel protein did not change during CD but this process increased kernel soluble proteins. The SDS-PAGE revealed relative increase of putative glutelins and vicilins with molecular weights of 19-24 and 41-58 kDa, respectively, in the kernel soluble protein fraction during CD, suggesting their enhanced solubility (Shahmoradi et al., 2013). Labeling with mBBr furthermore showed that glutelins are in a reduced state during the mobilization process. In addition, after SDS-PAGE zymography of kernel soluble proteins, a protease with a molecular weight of 80 kDa, showed more activity in aged kernels with higher MC. Kernel aging was also accompanied by the accumulation of total amino acids, proline and protein carbonylation especially at the moisture level of 20 % MC. Therefore, the occurrence of limited proteolysis in the seed during CD is very likely. Here, proteins with higher molecular weight but lower solubility are mobilized into polypeptides with slightly lower molecular weight but greater solubility (Shahmoradi et al., 2013), and therefore, no significant change of total protein is observed. Increased solubility of seed storage proteins makes them more susceptible to subsequent proteolysis (Kobrehel et al., 1992). Also, declined seed germination in wheat (Calucci et al., 2004; Galleschi et al., 2002) and Arabidopsis (Viñegra de la Torre et al., 2019) during CD is associated

with the increased protease activities, so it can play a role in the overall mobilization of proteins and leads to the release of amino acids (Palma et al., 2002). In sum, our results support the first hypothesis of this study, in which aging of walnut kernels e.g., by CD results in the mobilization of protein reserves.

Although PMSF pre-treatment could not improve the germination of kernels after CD, in other words, it was not physiologically efficient, but at the biochemical level it showed some signs of stress alleviation, most significantly, lower contents of proline and protein carbonylation following aging. Accumulation of proline in plants after exposure to environmental stress has been reported (Zhang and Becker, 2015). It has also been reported that during stressful conditions such as aged seeds, carbonyl derivatives including carbonyl proteins are accumulated (Ciacka et al., 2020; Dębska et al., 2013). Altogether, although the obtained data indicate the occurrence of mobilization of storage proteins in walnut kernels during CD, it does not support the hypothesis that the inhibition of storage proteins mobilization improves kernel tolerance to aging conditions. Indeed, as walnut kernels have others proteases (Chen et al., 2022) which are not inhibited by PMSF, pretreatment of kernels with protease inhibitors of a wider range of proteolytic enzymes might be promising to increase tolerance of kernels against aging conditions.

Conclusions

In this study, germination and biochemical data were used to understand the importance of proteolytic processes in the deterioration process of walnut kernels. The results showed that aging led to the mobilization of kernel storage proteins, however, the inhibition of storage protein mobilization, unlike lipids, was not efficient to prevent kernel aging. This may be due to storage proteins, compared to oils, are a minor fraction of kernel reserves in walnut, and hence the impact of their catabolism on kernel physiology would be minor. Alternatively, due to diverse proteases involved in storage protein mobilization in walnut kernels, pre-treatment of kernels with a wider range of protease inhibitors may improve tolerance to aging conditions. Undoubtedly, identification of metabolic pathways activated during walnut kernel aging can provide a comprehensive image of deterioration mechanism in this economic nut and leads to introduction of protocols for maintaining quality and viability of kernels during storage.

Keywords: Carbonylation, Controlled deterioration, Germination, Protease, Storage proteins, Walnut.

Acknowledgement

This research was supported by: Ph.D. Student project SFF by Iran National Science Foundation (INSF Grant No 98024880).

Declaration of conflict of interest

The authors declare no conflict of interests.