



## Effect of reactive oxygen species on germination and lipid proxidation in sunflower seeds

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### ABSTRACT

Reactive oxygen species cause to release of dormancy in many plants such as sunflower seeds. This study investigated in order to evaluation role of reactive oxygen species germination and lipid proxidation in sunflower seeds. This study was performed in two separate experiments, each in a completely randomized design with factorial design with four replications. In both experiments, uses from dormant and non dormant seeds of sunflower. It also applies of treatments Methylviologen and Cyanide in dormant seeds which are the producers of reactive oxygen species. Finally, germination lipid proxidation were evaluated as well. The results showed that the main reason for release of sunflower seeds dormancy is production of reactive oxygen species is an acceptable level so that seed germination of dormant seeds which was treated with Methylviologen and Cyanide was more than dormant control seeds and was similar to non dormant seeds. The amount of lipid peroxidation product malondialdehyde in dormant seeds was less than non dormant seeds and seeds treated with Methylviologen and Cyanide.

**Keywords:** lipid peroxidation, dormancy and reactive oxygen species

### INTRODUCTION

sunflower (*Helianthus annus* L.) is a plant of the *Asteraceae* family. This product Mexican areas of native vegetation that is America has been domesticated around 1000 years BC. Planting sunflower oil production and consumption of nuts has risen dramatically (Khaje poor, 2004 and Leubner-Metzger, 2005). Sunflower is one indifferent towards the day but needs a lot of light. Ratio is relatively resistant to soil salinity. Forage legumes in crop rotation, usually after the first weeding the crop is planted with plants that have a common root diseases such as peas, sugar beets and potatoes, are not included in the frequency. With sunflower, soybean, canola, cottonseed and peanut oil a year, which is the most important crop plants, it has long been an important part of agriculture is formed Eastern countries (Alyaree and Shekari, 2000).

The dual role of ROS in the physiological behavior of the seeds play. On the one hand, the activation of cellular signaling pathways act and the other as the products are known toxins that are produced under stress. Frequently read in various sources that the old reactive oxygen species Reactive Oxygen Species or ROS briefly toxic molecules that damage produced by stress conditions in plants and seed production is a sign of oxidative stress. But today is proved that the active oxygen species, despite the damaging effects they have useful roles in the body are living things. Organelles such as chloroplasts, mitochondria and oxidative activity proxies zoom or ultra-high-speed electron flow are the major source of ROS in plant cells (Gay et al, 1991; Giannopolitis et al, 1977; Gill et al, 2010 and Grant et al, 2000). In seed physiology (ROS) are generally regarded as toxic molecules are resulted in the accumulation of cell damage and impaired developmental processes of germination or sprouting. Crucial role of these compounds in the seeds of today's age has been well established (McDonald, 1999 and Moller, 2002). It has been recognized that ROS expression of some genes and signal transduction pathways that affect showing that the cells grow some strategy to take advantage of the ROS as stimulating the biological signal and to which the application of genetic stress answers to activate or control (Dalton, 1999) Recently, it has been found that plants actively produce ROS that there may be many different physiological processes such as biological stress response non biological defense against disease and signal to control systemic formation (Gill et al, 2010 ). There is evidence that suggests that ROS play a key role in seed germination and suggested that the cell wall loosening in the context of a growing contributes to the (Liszkay et al, 2004 and Luck, 1962). The aim of this study was to investigate the role of reactive oxygen species on germination and lipid proxidation sunflower seeds.

## MATERIALS AND METHODS

this research was carried out in order to study on effect of reactive oxygen species on sunflower seed germination and dormancy of Record cultivar using research budget of Young Researchers Club, Islamic Azad University Borojerd branch, Iran.

### Experiment 1

The experimental design was completely randomized with four replications. Sunflower seeds are harvested at a dormancy they need to after-ripening. For apply the after-ripening, dormancy a bunch of seed them for two months and were incubated at 60% relative humidity dormancy them to be broken. The treatments consisted of non dormant seed, dormant seeds, dormant seeds treated with methylviologen (producing reactive oxygen species) and dormant seeds treated with hydrogen cyanide (gas producing reactive oxygen species ) treatments. For germination test 25 seed petri-dishes on filter paper 9 cm (Top of paper) in foer replication. Seed germination tests were conducted in the dark and at a temperature of 25 degrees Celsius. Seeds were checked twice a day for 10 days and the number of germinated seeds were recorded. Seed germination, root exclusion based on the amount of 2 to 3 mm.

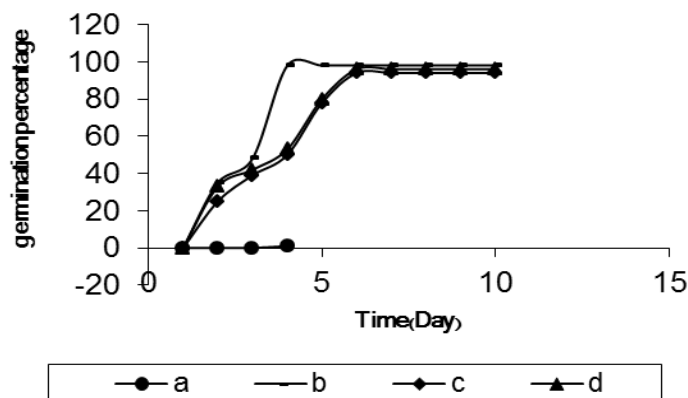
### Experiment 2

The experimental design was completely randomized with four replications. The treatments consisted of non dormant seed, dormant seeds, dormant seeds treated with methylviologen (producing reactive oxygen species) and dormant seeds treated with hydrogen cyanide (gas producing reactive oxygen species). Measurement of lipid peroxidation Heath and Packer (1968) method with changes were made . In this way, 0.25 g sunflower seeds with 5 ml of trichloroacetic 0.1 % of the Chinese mortar was crushed and homogenized . Homogenized extracts were transferred into centrifuge tubes for 30 min at 20 ° C. The speed of the centrifuge was g 16000 . The middle phase of the lipid substrate was used to measure lipid

peroxidation . 250 ml of the extract, 2 ml of reagent TBA 0.25 per cent were under 30 minutes in a boiling water bath . Immediately after this step, the test tubes were placed in ice for 15 minutes in a pan. After this period , the solution again for 10 min at 20°C were centrifuged and the amount of light-absorbing g16000 speed samples at wavelengths of 440, 532 and 600 nm was read . Blank solution containing 250 ml trichloroacetic 0.1, respectively , with 2 ml of reagent TBA 0.25 per cent were mixed , and all treatments were compared with the solution Blanc . Lipid peroxidation (LP) based on the amount of malondialdehyde (MDA) were determined. The statistical analyses to determine the individual and interactive effects of time cultivation and weeds control methods were conducted using JMP 5.0.1.2 (SAS Institute Inc., 2002). Statistical significance was declared at  $P \leq 0.05$  and  $P \leq 0.01$ . Treatment effects from the two runs of experiments followed a similar trend, and thus the data from the two independent runs were combined in the analysis.

## RESULTS AND DISCUSSION

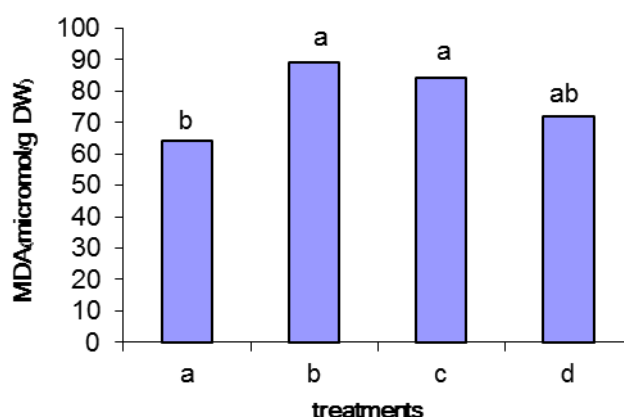
The results showed that different treatments within 5 to 7 days to reach maximum germination. The lowest germination was observed in dormant seeds so that after ten days of germination tests, germination was only 12%. Between non dormant seeds and seeds treated with methylviologen and cyanide these significant differences were observed among the three treatments, although the highest germination treatment without dormancy and 98% were achieved. After treatment without dormancy, seeds treated with cyanide (96%) and seeds treated with methylviologen (94%) had the highest rate of germination (Fig 1). Reactive oxygen species cause dormancy release of sunflower seeds are such that the production of reactive oxygen species during germination as a new mechanism known to release of dormancy and sunflower seed germination were introduced. Around the time of sunflower seeds in the presence of methylviologen 0.1mM seed dormancy efficiently remedied. These events can occur while the incubation of sunflower seeds with methylviologen cause particular purpose in dormant seeds and carbonilation proteins occurs in them only (Oracz et al, 2007). Sunflower seeds and embryonic axes within the cell cytoplasm, staining was observed and the amount of peroxide stains was very higher in non dormant seeds than dormant seeds (Oracz et al, 2007).



**Figure 1.** Germination percentage in different treatments.

A= dormant seeds B= non-dormant seeds C= dormant seeds treated with Methylviologen D= dormant seeds treated with Cyanid

As a result of lipid peroxidation is product of malondialdehyde. Effect of active oxygen species induced effects on lipid peroxidation and removing of seeds dormancy is malondialdehyde produced. This study was also observed in dormant seed production malondialdehyde (64.3 micromoles per gram dry weight of seeds) is less than that of the non dormant seeds and seeds treated with methylviologen and cyanide the terms of the There was a significant difference. Most of malondialdehyde production (89.25 micromoles per gram dry weight of seed) was produced in the seeds of non dormant terms of malondialdehyde production in seeds treated with methylviologen (84.25 mol g wet weight Dry seeds) and cyanide (72.1 micromoles per gram dry weight of seed), there was no significant difference Seeds stored dry spontaneously and non-enzymatic oxidation of lipids, which leads to the result that the MDA content significantly increases (figure 2).



**Figure 2.** MAD content in different treatments.

A= dormant seeds B= non-dormant seeds C= dormant seeds treated with Methylviologen D= dormant seeds treated with Cyanid

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