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Study on germination of sunflower seeds that affected by ROS

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ABSTRACT

This study investigated in order to evaluation of relationship between germination and produce of reactive oxygen species 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, the content of reactive oxygen species such as hydrogen peroxide and superoxide anion production 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. Also, production of Hydrogen peroxide and superoxide anion production of seeds treated with Methylviologen and Cyanide was similar to non dormant seeds and they had nearly doubled of dormant seeds.

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1. Introduction

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 disease 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). 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 in the regulation of dormancy and germinating in sunflower seeds.

2. 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 Rekord 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 methylviologene (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).

To extract hydrogen peroxide 0.2 g of seed samples with 3 ml tri chloro acetic acid, 0.1% and the porcelain mortar homogenized and extracted at 15,000 rpm for 15 min at 4°C centrifuge and the resulting extract was used to measure hydrogen peroxide. Active measurement was laid out as Jiazdwska et al (2010).

Superoxide radical content using Elstner and Hapel (1976) method was measured . In this method, 0.2 g fresh weight of sunflower seed axes in 4 ml of 50 mm sodium phosphate buffer with a ph of 7.8 and at a temperature of 4°C was solved . The resulting solution for 15 min and centrifuged at 15,000 rpm superoxide anion was used to estimate the resulting. To estimate superoxide anion ml of the extract obtained at 25 ° C for 30 min in the presence of hydroxylamine hydrochloride mm sodium phosphate buffer 50 mm with a ph of 7.8 data and incubated . Then 0.5 ml of it with a 5/ 0 ml of 17 mm sulfanilamide and 0/5 ml of 2 - Naftil amine 7 mm at 25°C for 30 min and incubated the samples were then centrifuged for 10 min at 13,000 rpm and the absorbance of the

sample at 540 nm was read . Standard curves were plotted with the use of sodium nitrate . The results in terms of micromoles per gram dry weight of seed samples were estimated (Oracz et al, 2007).

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.

3. Results and discussion

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).

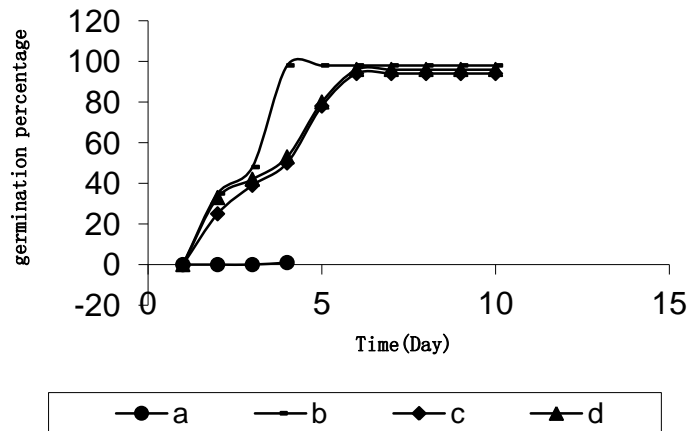


Fig. 1. Germination percentage in different treatments.

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

Production of reactive oxygen species such as hydrogen peroxide during the occurrence of seed germination can be changed. The results showed that hydrogen peroxide produced fewer seeds than dormant seeds and dormant seeds treated with methylviologen and cyanid. Hydrogen peroxide production in non dormant seeds about 1.11 micromoles per gram dry weight of the seed, which is about half the amount of seed material non dormant (2.45 micromoles per gram dry weight of seed) and between them in terms of There was no significant difference. Peroxide levels measured in the seeds treated with methylviologen (2.4 micromoles per gram dry weight of seed) and cyanide (2.33 micromoles per gram dry weight of seeds), roughly the same amount of hydrogen peroxide was produced in the dormant seeds. There was no significant difference between them in terms of the amount of hydrogen peroxide produced in the dormant seeds were no significant differences (Fig 2). 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).

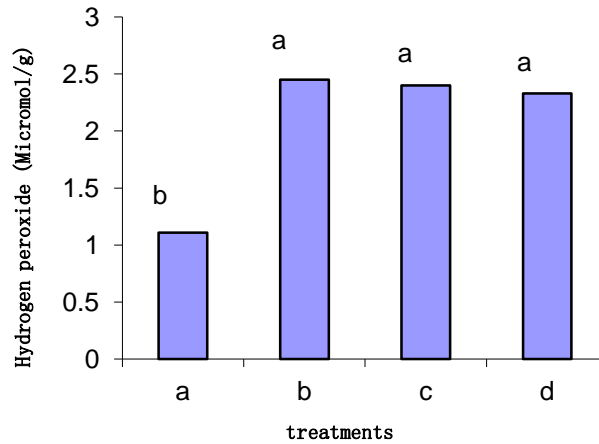


Fig. 2. H₂O₂ content in different treatments.

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

Dormant seeds than non dormant seeds and seeds treated with the superoxide anion production of methylviologen and cyanide production of superoxide anion was less, so that the dormant seeds (4.28 micromoles per gram dry weight of seed) was about half the rate of its production to other treatments There was a significant difference of opinion between them. In non dormant seed treatments and seed treated with methylviologen and cyanide, this difference was not significant, although the highest rate of superoxide anion production rate (9.2 micromoles per gram dry weight of seed) of non dormant seed (Fig 3). Formazan accumulation in seed axes dormant and within cells is not visible, but the seeds that indicate the presence of dark spots non dormant of superoxide anion has been observed (Oracz et al, 2007).

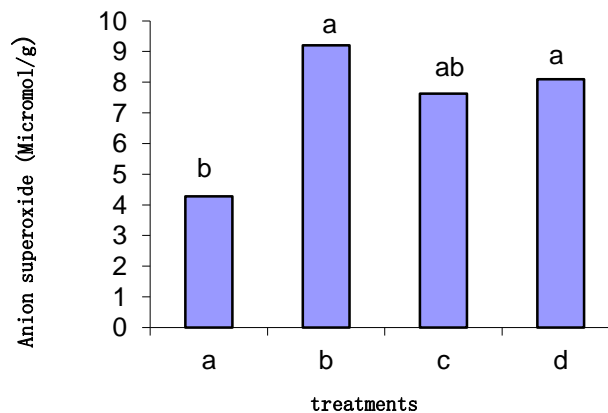


Fig. 3. Superoxide anion content in different treatments.

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

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