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## Assessment Production of Natural Reactive Oxygen Species Affected on Dormancy Alleviation, Germination and Antioxidant System in Sunflower Seeds

Mahdi Shaaban<sup>1\*</sup> Esfandiar Hassani Moghaddam<sup>2</sup> Ezatollah Nabati<sup>3</sup> and Zahra Rahmati Motlagh<sup>1</sup>

1- Young Researchers and Elite Club, Boroujerd Branch, Islamic Azad University, Boroujerd, Iran.

2- Seed and Plant Certification Research Institute (SPCRI), Agricultural Research Education and extension organization (AREEO), Karaj, Iran.

3- Lorestan Agricultural Science and Natural Research Organization, Boroujerd Station, Iran.

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### RESEARCH ARTICLE

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### ABSTRACT

**BACKGROUND:** The active oxygen species, despite the damaging effects they have useful roles in the body are living things.

**OBJECTIVES:** This research was done to determine whether Reactive Oxygen Species (ROS) could mediate Cyanide and Methylviologen signal in seed dormancy alleviation and sunflower seed germination, more widely, to assess their putative role in the control of seed germination and antioxidant system.

**METHODS:** The experiment was laid out in completely randomized design with four replications. The treatment consisted 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). For germination test 25 seed take in 9cm petridishes on filter paper. For biochemical assay seeds were imbibed for 24h and therefore Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), Malondialdehyde (MDA) and four antioxidant enzymes such as Catalase (CAT), Proxidase (POX), Ascorbat proxidase (APX) and Superoxide dismutase (SOD) were measured.

**RESULT:** Maximum seed germination was recorded at non-dormant seeds and minimum of it was measured at dormant seeds. Lowest H<sub>2</sub>O<sub>2</sub> and MDA production and lowest APX activity was recorded at dormant seeds. However, highest three detoxified enzymes activities such as CAT, POX and SOD was founded at dormant seeds. This enzyme activity was coinciding with lowest ROS production such as H<sub>2</sub>O<sub>2</sub> and MDA production. ROS production as resources such as Cyanide and Methylviologen play a key role in the control of sunflower dormancy alleviation and seed germination.

**CONCLUSION:** Main reason for seeds dormancy alleviation is production of ROS is acceptable level so germination of dormant seeds which was treated with Methylviologen and Cyanide was more than dormant control seeds and was similar to non-dormant seeds.

**KEYWORDS:** *Catalase, Cyanide, Malondialdehyde, Super oxide dismutase.*

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## 1. BACKGROUND

The seeds of most angiosperms are dormant at maturity, and dormancy must be lost before germination can occur (Bewley, 1997). This pause in the plant life cycle allows germination to occur under conditions favorable for growth of the seedling and in a season that provides sufficient time for completion of the next generation. Dormancy is a property of an intact seed, but several parts within the seed can contribute to seed dormancy (Bewley, 1997; Koornneef *et al.*, 2000). Seed germination incorporates events that commence with the uptake of water (imbibition) by the quiescent dry seed and terminates with the emergence of the embryonic axis, usually the radicle (Bewley, 1997). Frequently read in various sources that the old 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. Reactive oxygen species, including the superoxide anion radical, hydrogen peroxide, the hydroxyl radical, and singlet oxygen, are metabolic by products in both plants and animals. Although the toxicity of ROS is well documented, cellular antioxidant mechanisms seem to control ROS concentrations tightly, rather than to eliminate them completely, suggesting that some ROS play physiological roles and act as signaling molecules (Bailly, 2004). 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; Grant *et al.*, 2000). In seed physiology ROS are generally regarded as toxic molecules are resulted in the ac-

cumulation 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; 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; Luck, 1962). Bailly (2004) told that seed germination and post-germination seedling development are well-regulated process in plant physiology that involving high metabolic activity and generation of reactive oxygen species (ROS) in the plant cells. ROS affect dual role in seed physiology, displaying two major functions: as a kind of cytotoxin and as a special role in seed development, dormancy breakage, and in defense against biotic and abiotic stresses (Apel and Hirt, 2004). The effect of Cyanide (HCN) in releasing seed dormancy has been demonstrated mainly for cereals and to a lesser extent for members of other plant families, such as the Asteraceae and Rosaceae (Roberts, 1973; Esashi *et al.*, 1979; Bogatek and Lewak, 1988). Oracz *et al.* (2007, 2008) recently proposed that a short-term treatment (about 3h) with gaseous Cyanide

could act as a signal involved in the alleviation of embryo dormancy in sunflower seeds. Oracz *et al.* (2009) told that dormancy breaking by Cyanide could be a consequence of reactive oxygen species accumulation in seeds. In dormant sunflower seeds Cyanide triggered the expression of the transcription factor Ethylene Response Factor1, thus suggesting a cross talk between ethylene and Cyanide pathways that laid to dormancy breaking (Oracz *et al.*, 2008). ROS defense network, composed of antioxidant enzymes, antioxidants and ROS producing enzymes, is responsible for maintaining ROS levels under tight control. In plant cells, antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT), are considered to form a defensive team, whose combined purpose is to protect cells from oxidative damage (Blokhina *et al.*, 2003). Moreover, malondialdehyde (MDA) is considered sensitive marker commonly used for assessing membrane lipid peroxidation (Bailly *et al.*, 1996; Goel and Sheoran, 2003). Leymarie *et al.* (2011) in Arabidopsis showed that activities of some detoxifying enzymes such as CAT, SOD and GR decreased in non dormant seeds.

## 2. OBJECTIVES

This research was done to determine whether ROS could mediate Cyanide and Methylviologen signal in seed dormancy alleviation and sunflower seed germination, more widely, to assess their putative role in the control of seed germination and antioxidant system.

## 3. MATERIALS AND METHODS

### 3.1. Field and Treatments Information

This research was carried out to study effect of reactive oxygen species (ROS) source (Cyanide and Methylviologen) on sunflower (cv. Record)

dormancy alleviation, seed germination and biochemical details according completely randomized design with four replications with using research budget of Young Researchers and Elite Club, Islamic Azad University, Boroujerd branch, Boroujerd Iran, in 2013. The treatment consisted was in four levels such as 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) or 3h before germination test and biochemical assay.

### 3.2. Farm Management

Sunflower seeds are harvested at maturity and dormancy alleviation for seeds is need by after-ripening. For apply the after-ripening of seeds and dormancy alleviation seeds were incubated at 60% relative humidity for two months. For germination test 25 seed take in 9 cm petridishes on filter paper (Top of paper) in four replications. Seed germination tests were conducted in the dark and at a temperature of 25°C Celsius. Seeds were checked twice a day for 10 days and the number of germinated seeds was recorded.

### 3.3. Measured Traits

For biochemical assay seeds were imbibed for 24h and therefore hydrogen peroxide, MDA and four antioxidant enzymes such as catalase (CAT), peroxidase (POX), ascorbat peroxidase (APX) and superoxide dismutase (SOD) were measured. To extract hydrogen peroxide 0.2 g of seed samples with 3 ml trichloro 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). MDA content was deter-

mined by the thiobarbituric acid (TBA) reaction (Bailly *et al.*, 1996). Endosperms or cotyledons were homogenized with 0.1% trichloroacetic acid (TCA) (m/v, 1/10) and the homogenates were centrifuged at 15000×g for 15 min. To a 1.0 mL aliquot of the supernatant, 3.0 mL of 0.5% TBA in 5% TCA was added. The mixture was heated at 95°C for 30 min and then cooled immediately in an ice bath. The reaction mixture was centrifuged at 15000×g for 10 min and the observance of supernatant was recorded at 532 nm and 600 nm. Lipid peroxidation was expressed as MDA content in  $\mu\text{M}$  per gram fresh weight, by using an extinction coefficient of  $155 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ . For the catalase (CAT) assay, soluble proteins were extracted by homogenizing 0.1 g (fresh weight) powdered sample in 3 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM ethylenediaminetetra acetic acid (EDTA) and 1% polyvinyl pyrrolidone, with the addition of 1 mM guaiacol and ascorbic acid in the case of proxidase (POX) and ascorbate peroxidase (APX) assays respectively. CAT activity was measured following the change in the absorbance of the reaction mixture at 240 nm (Philippe *et al.*, 2007). The assay was detected in a 3 ml reaction mixture containing 2.8 mL phosphate buffer (50 mM, pH 7.0), 100  $\mu\text{L}$   $\text{H}_2\text{O}_2$  (1%) and 100  $\mu\text{L}$  of crude extract. POX activity was measured by Sakharov and Aridilla (1999) method with slight modifications. A 3 mL mixture consisted of 2.85 mL of guaiacol (3%), 0.1 mL  $\text{H}_2\text{O}_2$  (2%) and 50  $\mu\text{L}$  enzyme extract and the

changes of absorbance at 470nm were measured using a UV/vis spectrophotometer. One unit of POX activity was expressed as 1.0 change in absorbance per minute. Total APX as Nakano and Asada (1981) in a 3-ml total volume. SOD activity was determined by the method of Chen and Pan (1996). The reaction mixtures included 50 mM sodium phosphate buffer (pH 7.0), 10 mM methionine, 1.17 mM riboflavin, 56 mM NBT and 100  $\mu\text{L}$  protein extract. The changes of absorbance were read at 560 nm using a UV/vis spectrophotometer. One SOD unit was defined as the enzyme activity that reduced the photoreduction of nitroblue tetrazolium to blue formazan by 50%.

### 3.4. Statistical Analysis

The statistical analyses to determine all traits were conducted with 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.

## 4. RESULTS AND DISCUSSIONS

The results of analysis of variance shoes that the effect of treatment was significant on seed germination, hydrogen peroxide, Malondialdehyde, Catalase activity, Proxidase activity, Ascorbat peroxidase activity and Superoxide dismutase activity (Table 1). Results showed that between dormant seeds and other significant differences were observed.

**Table 1.** ANOVA effect of ROS resources on germination and enzyme activity in sunflower seeds

S.O.V	df	Seed germination	Hydrogen peroxide	Malondialdehyde	Catalase
Treatment	3	2433**	22413*	1356**	892.6*
Error	12	26	225.5	24.2	34.4
CV (%)	-	6.5	9.9	7.5	3.4

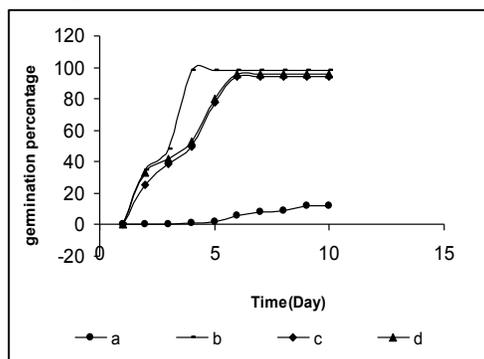
<sup>ns</sup>, \*\* and \*: non-significant, significant at 1% and 5% probability level, respectively.

Continue Table 1.

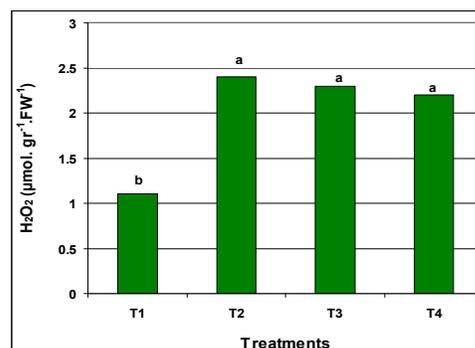
S.O.V	df	Proxidase	Ascorbat oxidase	Superoxide dismutase
Treatment	3	227.7**	173.4**	2.14*
Error	12	91.2	19.4	0.024
CV (%)	-	3.8	9.2	6.13

<sup>ns</sup>, \*\* and \*: non-significant, significant at 1% and 5% probability level, respectively.

Maximum seed germination percentage (98%) was recorded at non-dormant seeds. After 10 days germination test at 25°C seeds treated with Cyanide had highest seed germination (96%) after non-dormant seeds. Germination percentage in seeds treated with Methylviologen (94%) was more than germination percentage at dormant seeds treatment but was less than other treatments (Fig. 1). Production of ROS such as H<sub>2</sub>O<sub>2</sub> during occurrence of seed germination can be changed. Fig. 2 shows the changes in the ability of non-dormant seeds and seeds treated by Cyanide and Methylviologen to produce H<sub>2</sub>O<sub>2</sub>, which was measured in the incubation medium, during 24 h of imbibition at 25°C. H<sub>2</sub>O<sub>2</sub> production in non-dormant seeds and seeds treated by Cyanide and Methylviologen was almost similar and close to 2.4 μmol.gr<sup>-1</sup>. FW<sup>-1</sup> (Fig. 2). The results showed H<sub>2</sub>O<sub>2</sub> production in dormant seeds was less than non-dormant and seeds treated with Methylviologen and Cyanide.



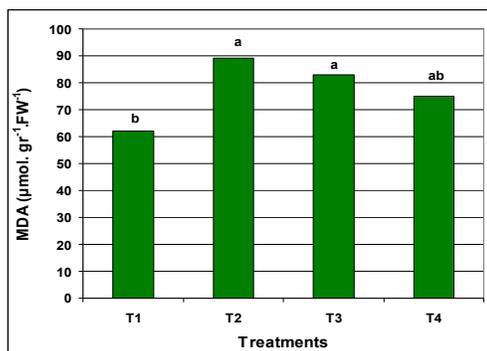
**Fig. 1.** Germination percentage affected different treatments. A= dormant seeds, B= non-dormant seeds, C= dormant seeds treated with Methylviologen, D= dormant seeds treated with Cyanide.



**Fig. 2.** Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content affected different treatments. T<sub>1</sub>= dormant seeds, T<sub>2</sub>= non-dormant seeds, T<sub>3</sub>= dormant seeds treated with Methylviologen, T<sub>4</sub>= dormant seeds treated with Cyanide.

Hydrogen peroxide production in dormant seeds after 24 h imbibition in 25°C was 8.5 μmol.gr<sup>-1</sup>. FW<sup>-1</sup> but, in non-dormant seeds was 2.4 μmol.gr<sup>-1</sup>. FW<sup>-1</sup> that was higher rate than other treatments. Excepting dormant seeds that produced minimum hydrogen peroxide between other treatments the differences were not significant (Fig. 3). The changes of MDA content in treated and untreated seeds during dormancy breaking and germination of sunflower are shown in Fig. 3. MDA contents in non-dormant seeds and seeds treated by Cyanide and Methylviologen increase gradually during the 24h imbibition at 25°C. In non-dormant seeds, MDA content increased sharply during the first 24h of imbibition and then. However MDA production in non-dormant seeds after 24 h imbibition in 25°C was 89 μmol. gr<sup>-1</sup>.FW<sup>-1</sup>. MDA production in seeds treated by Methylviologen after 24 h imbibition in 25°C was 70 μmol. gr<sup>-1</sup>.FW<sup>-1</sup> but in seeds treated by Cya-

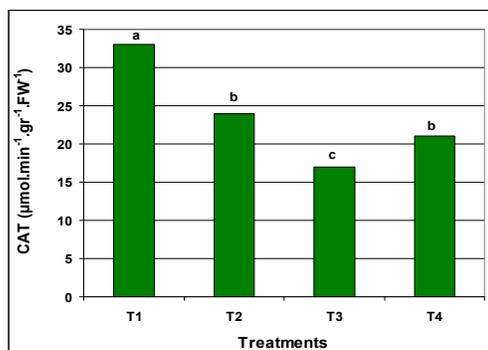
nide was  $82 \mu\text{mol. gr}^{-1}.\text{FW}^{-1}$  that their difference was not significant with non-dormant seeds. Moreover, in dormant seeds that was minimum MDA production after 24 h imbibition in  $25^{\circ}\text{C}$  was  $63 \mu\text{mol. gr}^{-1}.\text{FW}^{-1}$  MDA production was recorded (Fig. 3).



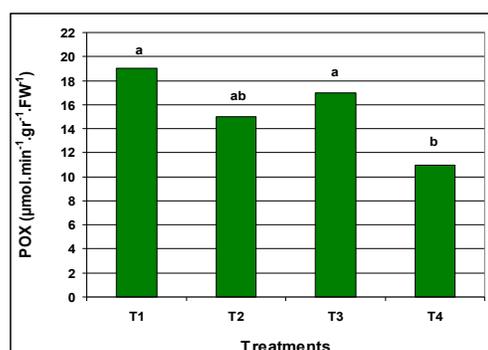
**Fig. 3.** Malondialdehyde (MDA) content affected different treatments. T<sub>1</sub>= dormant seeds, T<sub>2</sub>= non-dormant seeds, T<sub>3</sub>= dormant seeds treated with Methylviologen, T<sub>4</sub>= dormant seeds treated with Cyanide

Activity of CAT, POX and APX was determined in vitro in the presence of various concentrations of Cyanide and Methylviologen and in dormant and non-dormant seeds. Maximum CAT activity was founded at dormant sunflower seeds. However CAT activity in dormant seeds after 24 h imbibition in  $25^{\circ}\text{C}$  was  $33 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$ . After that non-dormant seeds and treated seeds by Methylviologen had CAT activity more than seeds treated by Cyanide. CAT activity in seeds treated by Cyanide after 24 h imbibition in  $25^{\circ}\text{C}$  was  $16 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$  that was minimum activity. In seeds treated by Cyanide CAT activity was 50% less than dormant seeds (Fig. 4). The changes of POX activities in treatments during dormancy alleviation and germination are shown in Fig. 5. POX activities in dormant seeds were more than other treatments. In dormant seeds POX activity after 24h imbibition in  $25^{\circ}\text{C}$  was

$19 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$ . POX activity in seeds treated by Cyanide after 24h imbibition in  $25^{\circ}\text{C}$  was  $17.6 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$  that difference of it with dormant seeds was not significant. POX activity in non-dormant seeds after 24h imbibition in  $25^{\circ}\text{C}$  was  $16.2 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$  that was more than seeds treated by Methylviologen. However, in seeds treated by Methylviologen POX activity after 24h imbibition in  $25^{\circ}\text{C}$  was  $11.3 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$  that was minimum POX activity in all treatments (Fig. 5). The results showed that APX activity was higher in non-dormant seeds rather than other treatments. However APX activity in non-dormant seeds after 24h imbibition in  $25^{\circ}\text{C}$  was  $12.2 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$ . Minimum APX activity was recorded for dormant seeds that were  $6 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$  after 24h imbibition in  $25^{\circ}\text{C}$ . APX activity in non-dormant seeds was tow times more than dormant seeds. However APX activity in seeds treated by Methylviologen was more than seeds treated by Cyanide as after 24h imbibition in  $25^{\circ}\text{C}$  was  $8.3 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$  but, their difference was not significant (Fig. 6). The results showed that SOD activity was higher in dormant seeds rather than other treatments. SOD activity in dormant seeds after 24 h imbibition in  $25^{\circ}\text{C}$  was  $11 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$  and in non dormant seeds was  $5 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$  and difference was significant. The seeds treated by Methylviologen had SOD activity more than Cyanide treated seeds, so that their activity after 24 h imbibition in  $25^{\circ}\text{C}$  was  $8.5 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$  but SOD activity in seeds treated by Cyanide was  $6.5 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$  at the same condition. Minimum SOD activity after 24h imbibition in  $25^{\circ}\text{C}$  was  $5.2 \mu\text{mol. min}^{-1}.\text{gr}^{-1}.\text{FW}^{-1}$  in non-dormant seeds (Fig. 7).



**Fig. 4.** Catalase activity (CAT) affected different treatments. T<sub>1</sub>= dormant seeds, T<sub>2</sub>= non-dormant seeds, T<sub>3</sub>= dormant seeds treated with Methylviologen, T<sub>4</sub>= dormant seeds treated with Cyanide

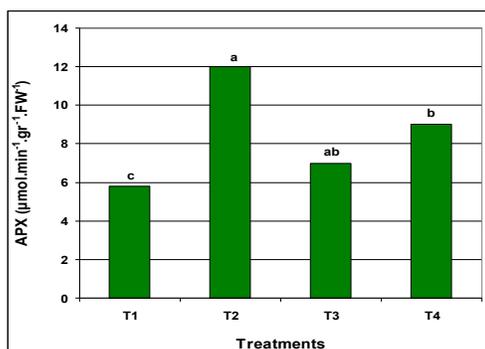


**Fig. 5.** Proxidase activity (POX) affected different treatments. T<sub>1</sub>= dormant seeds, T<sub>2</sub>= non-dormant seeds, T<sub>3</sub>= dormant seeds treated with Methylviologen, T<sub>4</sub>= dormant seeds treated with Cyanide

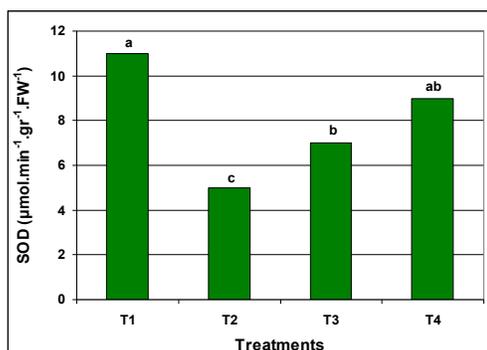
Dormancy release and germination process stimulate by ROS production Bewley and Black (1994) told that dormancy is well known to be a relative phenomenon that is controlled by environmental factors during seed imbibition, and its expression can vary greatly with hydration level, temperature, oxygen availability or light. In this study results showed that germination percentage in non-dormant seeds was same to Cyanide and Methylviologen treated seeds. However germination percentage in dormant seeds had a minimum rate (Fig. 1). 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). In seed biology, ROS also play a key role in seed dormancy alleviation, after-ripening, and germination (Oracz *et al.*, 2007). In a previous report, it was shown that KCN vapors effectively reduced Arabidopsis seed dormancy (Bethke *et al.*, 2006b). Reactive oxygen species cause dormancy alleviation 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. Cyanide and Methylviologen induced after-ripening in dormant seeds and dormancy was alleviated, therefore seeds became able to germinate fully in Cyanide and Methylviologen seed treated that in result their germination percentage were same to non-dormant and after-ripened seeds (Fig. 1). However, Cyanide and Methylviologen are able to release seed dormancy and increased germination percentage in a similar manner as observed for non-dormant seeds that had the maximum germination percentage (Fig. 1). Methylviologen (MV) gains electrons from reductants to form the Methylviologen cation radical, MV<sup>+</sup>, which reacts with ground state oxygen to produce O<sub>2</sub><sup>-</sup> (Calderbank and Slade, 1976; Oracz *et al.*, 2009). H<sub>2</sub>O<sub>2</sub> is one of the ROS compounds that produce during germination and dormancy alleviation in oxidative window and stimulate germination of non-dormant seeds. During dormancy release and seed germination of sunflower seeds H<sub>2</sub>O<sub>2</sub> production increased significantly. Seeds that treated by Cyanide and Methylviologen produced H<sub>2</sub>O<sub>2</sub> same to non-dormant seeds. However production of H<sub>2</sub>O<sub>2</sub> in dormant seeds was less than non-dormant seeds and Cyanide and

Methylviologen seeds treated (Fig. 2). In sunflower,  $H_2O_2$  produced in the presence of HCN, a dormancy release compound has been shown to activate downstream elements of the ethylene signaling pathway (Oracz *et al.*, 2009).



**Fig. 6.** Ascorbat proxidase (APX) activity in different treatments. T<sub>1</sub>= dormant seeds, T<sub>2</sub>= non-dormant seeds, T<sub>3</sub>= dormant seeds treated with Methylviologen, T<sub>4</sub>= dormant seeds treated with Cyanide



**Fig. 7.** Super Oxide Dismutase (SOD) activity in different treatments. T<sub>1</sub>= dormant seeds, T<sub>2</sub>= non-dormant seeds, T<sub>3</sub>= dormant seeds treated with Methylviologen, T<sub>4</sub>= dormant seeds treated with Cyanide.

Liu *et al.* (2010) suggested that  $H_2O_2$  reduces ABA synthesis and stimulates gibberellin synthesis, thus releasing dormancy, although the data in this study were obtained using exogenous  $H_2O_2$ , which does not reflect the *in vivo* situation. However in sunflower,  $H_2O_2$  produced in the presence of Cyanide, a dormancy release compound,

has been shown to activate downstream elements of the ethylene signaling pathway (Oracz *et al.*, 2009). In Arabidopsis, however, Leymarie *et al.* (2011) revealed that  $H_2O_2$  production in dormant and non-dormant seeds was almost similar. They told that, seed imbibition longer than 3 h in darkness was accompanied by a decreased production of  $H_2O_2$  in both dormant and non-dormant seeds. In this study we proposed that during germination of sunflower seeds ROS such as  $H_2O_2$  was produced and treated seeds by Cyanide and Methylviologen stimulate ROS production and dormancy alleviation, and then increased germination percentage. Germination is completed only when the ROS content is within an oxidative window that allows ROS signaling (Bailly *et al.*, 2008). They told that low or high amounts of ROS at above or below the 'oxidative window for germination would not permit progress towards germination. According to this model, seed dormancy, i.e. the inability of seeds to germinate in favorable environmental conditions (Finch-Savage and Leubner-Metzger, 2006), is regulated by ROS signaling. However, alleviation of sunflower seed dormancy during after-ripening is associated with ROS production which triggers oxidation of specific proteins and mRNA, thus altering cell signaling during subsequent seed imbibition of after-ripened seeds (Oracz *et al.*, 2007, Bazin *et al.*, 2011). The present study showed that the Cyanide and Methylviologen signal transduction are mediated by ROS and, consequently, that ROS play a key role in the dormancy release and starting of germination process. It is well known that during dormancy release and germination process ROS produced and therefore ROS induced lipid peroxidation of membranes that is a reflection of stress induced damage at the cellular

level. Our findings indicate that lipid peroxidation occurred during seed germination and early seedlings growth. The change in MDA contents is often used as an indicator of oxidative damage (Sung, 1996). In the present study increased MDA contents in non-dormant seeds and dormant seeds treated with Cyanide and Methylviologen during seed germination. Oracz *et al.* (2009) suggested that lipid peroxidation increases during germination process in sunflower seeds. In this study, we noted that MDA content increased in parallel with the increase in seed germination and  $H_2O_2$  content. Schopfer *et al.* (2001) told that elevated MDA contents mediated by free radicals and peroxides are considered to be one of the likely explanations for lipid peroxidation during germination. It is generally recognized that plants can protect themselves by inhibiting lipid peroxidation by activated antioxidant enzymes after imbibition (Bailly, 2004). In the present study activities of the main antioxidant enzymes, i.e. CAT, POX, APX and SOD were assessed in dormant and non-dormant seeds and seeds treated by Cyanide and Methylviologen. All antioxidant enzymes activities are expressed as a function of the activities measured in dormant and non-dormant seeds. Dormant seeds had the highest CAT, POX and SOD activities rather than other treatments. However dormant seeds had the lowest APX enzyme activity. Both treated seeds by Cyanide and Methylviologen had the medium behavior for CAT, POX, APX and SOD activities. Seeds treated by Cyanide and Methylviologen produced ROS that laid to the dormancy alleviation in sunflower dormant seeds that laid to the increases of seed germination. There was a trend for a higher activity of the three detoxifying enzymes such as CAT, POX and SOD in dor-

mant seeds after imbibition, and this increase was significant for above three detoxifying enzymes, which was markedly stimulated during seed imbibition that agreed this research. Leymarie *et al.* (2011) in Arabidopsis showed that activities of some detoxifying enzymes such as CAT, SOD and GR decreased in non dormant seeds. They told that there was a trend for a lower activity of the these three detoxifying enzymes in non-dormant axes after 24 h of imbibition at 25°C, but this decrease was significant only for CAT. ROS production such as  $H_2O_2$  in dormant seeds after 24h imbibition in 25°C was  $8.5 \mu\text{mol.gr}^{-1} \text{FW}^{-1}$  and was lowest rate in all treats. In dormant seeds both lowest ROS production and highest three detoxifying enzymes such as CAT, POX and SOD activities were recorded. Bailly (2004) told that ROS content increases during the early stages of seed imbibition, as a consequence of the resumption of respiration. However, this increase might occur very early or be masked by an efficient activation of scavenging or detoxifying mechanisms, such as CAT (Leymarie *et al.*, 2011). Oracz *et al.* (2009) told that Cyanide signal transduction is mediated by ROS and, consequently, that ROS play a key role in the germination process. In the present study non-dormant seeds and seeds treated by Cyanide and Methylviologen produced approximately twice more  $H_2O_2$  than dormant seeds 24h after imbibition (Fig. 2). It is possible that this decrease in  $H_2O_2$  was partly related to the increase of CAT, POX and SOD activity, which was demonstrated for dormant seeds imbibed for 24h. Leymarie *et al.* (2011) told that changes in detoxifying enzymes activities were related to an activation of the mechanisms involved in ROS production, since their method of  $H_2O_2$  measurement evaluates the ability to produce of  $H_2O_2$ .

## 5. CONCLUSION

In this study we demonstrated that natural ROS production mimicry as it resources such as Cyanide and Methylviologen play a key role in the control of sunflower dormancy alleviation and seed germination. However, our results showed that the main reason for sunflower seeds dormancy alleviation 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 MDA and three detoxified enzymes (CAT, POX and SOD) 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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