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Seed Deterioration in Eruca sativa (Miller) Thell. Varieties: Comparative factors between Seeds and Leaves

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Abstract— The present study focuses on the role of oxidative stress and the antioxidant defense mechanism in deteriorated seed of Eruca sativa (Miller) Thell. Fifteen varieties were undertaken to demonstrate the effect of seed deterioration in seeds and leaves and observations were recorded for seed germination and seedling growth parameters, and various biophysical and biochemical parameters. Increased level of electrolyte leakage, malondialdehyde and reactive oxygen species was observed in varieties with low germination percentage. A high level of correlation was found between the loss of seed viability and increase level of reactive oxygen species. Seed deterioration appears to be attributed to loss of antioxidant defense mechanism.
Keywords— Antioxidants, Eruca sativa, Malondialdehyde, Reactive Oxygen Species, Seed deterioration.

I. INTRODUCTION

Eruca sativa (Miller) Thell, known as taramira, arugula or rocket, belongs to Brassicaceae family, is an important oilseed crop, commonly distributed all over the world. The young plants are usually consumed as a salad, vegetable and as green fodder for its typical spicy taste [1]. It has varied medicinal and therapeutic properties including inhibition of tumorigenesis [2], hepatoprotective activities [3] and anti-ulcer [4]. The essential oil extracted from the leaves of *E. sativa* contains 67 volatile compounds which constitutes 96.52% of the oil [5]. The seed oil has various pharmacological efficacy and potential bio-active compounds as compared to different aerial and root plant extracts [6].

Seed deterioration can be defined as "downturn changes occurring with time increasing the seeds vulnerability to external challenges and decreasing the ability of the seed to survive". Seed deterioration results in loss of viability, vigor and overall seed quality due to aging or effect of adverse environmental factors [7,8]. Annual losses from seed harvests due to deterioration is recorded as much as 25% of the harvested crop [9]. It is one of the basic reasons for low productivity [10]**.** It has been described as cumulative, irreversible, degenerative and inexorable process [11]. The physiology of seed deterioration is a separate incident from seed development and germination. As seed deterioration increases, seed performance gradually decreases. Losses in seed quality occur during field weathering, harvesting and storage. The rate of deterioration fluctuates critically from one species to another and also among varieties of the same species [12]. Deterioration is evident as a reduction in percentage germination, loss of vigor, produce weak seedlings, loss of vigor, become less viable and eventually seed death [13]. The percentage emergence of deteriorated seeds is less than healthy seeds. Henceforth, deteriorated seed produces uneven stands, spotty fields, and fewer plants per hectare than healthy seed [8]. Plants that have originated from deteriorate seed can also reduce growth rate [7]. The present study focuses on the role of oxidative stress and the antioxidant defense mechanism in deteriorated seed of *Eruca sativa.*

II. MATERIALS AND METHODS

Fifteen varieties of *Eruca sativa* were secured from the Department of Plant Breeding & Genetics. S.K.N. College of Agriculture, Jobner. Evenly selected seeds were sterilized with 5 % sodium hypochlorite (NaClO) for 2 min and then repetitively washed under running tap water followed by distilled water. Nearly 10 seeds were sown in each Petri dish and incubated in BOD incubator set at 25°C. Seed germination parameters were computed regularly up to 5 days and seedling growth parameters were made after 5 days of sowing. Different biochemical traits were analysed after 6 days of sowing. Each treatment was replicated three times and the data represented as average values.

TABLE I

LIST OF FIFTEEN *E. SATIVA* VARIETIES

S. No.	Varieties	S. No.	Varieties
1.	RTM-1212	9.	RTM – 1035
2.	RTM-1358	10.	RTM – 1107
3.	RTM - 603	11.	RTM-314
4.	RTM – 2002	12.	RTM – 1359
5.	RTM – 673	13.	RTM – 1301
6.	T-27	14.	RTM – 1356
7.	RTM - 1310	15.	RTM- 1354
8.	RTM – 1351		

A. Seed Germination Parameters

1) Percentage Germination (%)

The percent germination was recorded daily up to five days. The seeds were taken as germinated when radical had emerged from the seed coat.

Percentage germination (%) =

$$\frac{\text{Number of germinated seeds} \times 100}{\text{Total number of seeds}}$$

2) Speed of Germination (SOG)

It is defined as the maximum daily germination reached at any time. Number of germinated seeds in each variety was counted daily and speed of germination was calculated as per the formula suggested by Maguire [14]:

$$SOG = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots\ldots + \frac{X_n - (X_n - 1)}{Y_n}$$

Where, X1, X2 and X_n = number of germinated seeds on 1st 2nd and nth day, respectively

Y1, Y2 and Y_n = number of days from sowing to 1st, 2nd and nth count, respectively.

3) Coefficient of Germination (COG) (%)

The coefficient of germination is an index of rapidity or the rate of germination of seeds. It was calculated using the formula [15]:

$$COG\ (\%) = \frac{A_1 + A_2 + \ldots\ldots\ldots A_n \times 100}{A_1T_1 + A_2T_2 + \ldots\ldots\ldots A_nT_n}$$

A = number of seeds germinated; T = time (days) corresponding to A; n = number of days to final count

4) Mean of Germination Time (MGT) [16] :

$$MGT = \frac{\Sigma d_i}{\Sigma n_i}$$

n_i = number of germinated seeds in every count; d_i = day of counting

B. Seedling growth parameters:

1) Root and Shoot length

Five normal seedlings were selected at random and used for measuring root and shoot length. Mean root and shoot length was expressed in centimeters.

2) Fresh and Dry matter

Five seedlings from each petri dish were taken and their fresh matter was recorded after 5th day. For Dry matter seedlings were oven dried at 80°C for 72 h. The dried seedlings were weighed to estimate the dry matter and the mean values were expressed in g.

3) Seedling Vigor Index (VI)

The seedling vigor index was computed by adopting the method suggested by Abdul-Baki and Anderson [17] and expressed as an index numbers.

Seedling Vigor Index = [Root length (cm) + Shoot length (cm)] × Germination (%)

C. Biophysical Parametres:

1) Relative Leaf Water Content (RLWC)

The RLWC was measured according to Barrs and Weatherley [18]. Percentage relative leaf water content was calculated as follows:

$$\text{RLWC (\%)} = \frac{\text{Fresh wt.} - \text{Dry matter}}{\text{Turgid wt.} - \text{Dry matter}} \times 100$$

2) Electrolyte leakage (EL)

Electrolyte leakage analysis was determined according to the method of Zhang *et al*., [19]. Percentage of electrolyte leakage was estimated form the equation:

EL (%) = (Xi / Xi + Xt) × 100

D. Biochemical Parameters:

1) Malonaldehyde (MDA)

The extent of lipid peroxidation was estimated in term of malondialdehyde (MDA) content, a product of lipid peroxidation, by the method described by Heath and Packer [20]. The absorbance was read at 535nm. MDA content was calculated by extinction coefficient of 155 $mM^{-1}cm^{-1}$ expressed as per gram of fresh weight.

2) Ascorbic Acid

AA was estimated by the method of Mukharjee and Chaudhuri [21]. The reaction mixture consist extract, 2% Dinitrophenylhydrazine (in acidic medium) and 1 drop of 10% thiourea (in 70% ethanol) and kept in boiling water bath for 15 min. The mixture was cooled at room temperature 80% (v/v) sulphuric acid (chilled) was added to the mixture. The absorbance at 530 nm was recorded.

3) Proline Content

The proline content was assessed by the method of Bates *et al.,* [22]. The reaction mixture consisting of supernatant, acid ninhydrin and glacial acetic acid was boiled at 100°C for 1 h to develop colour. After termination of the reaction in ice bath, the reaction mixture was extracted with toluene and the absorbance was read at 520 nm against toluene as a blank.

4) Ascorbate Peroxidase (APX)

APX activity was determined based on the oxidation of ascorbate as a decrease in absorbance at 290 nm [23]. For the estimation reaction mixture containing 50 mM phosphate buffer (pH 7.0), 0.1 M EDTA, 0.5 mM ascorbic acid and enzyme extract. The

reaction was started with the addition of 0.1 mM hydrogen peroxide. The decrease in absorbance for a period of 30 s was measured at 290 nm after addition of H_2O_2. The rate constant was calculated using the extinction coefficient of 2.8 mM^{-1} cm^{-1}.

5) Catalase (CAT)

Catalase activity was determined by the method of Aebi [24]. The reaction was initiated by adding H_2O_2 to enzyme extract and absorbance was recorded at 410 nm for 2 min. The reaction was initiated by adding the enzyme solution. It was expressed as µM $ml^{-1}min^{-1}mg^{-1}$ protein.

6) Superoxide Dismutase (SOD)

The activity of SOD was estimated by the method of Dhindsa *et al.,* [25]. The photo-reduction of NBT resulted in the formation of purple formazon. The absorbance was read at 560 nm and the total SOD activity of the sample was estimated by measuring its ability to inhibit the photochemical reduction of nitro-blue-tetrazolium (NBT). The activity of SOD is expressed as change in OD $min^{-1}ml^{-1}mg^{-1}$ protein.

7) Pyrogallol Peroxidase (PPX)

PPX activity was assayed by the method of Kar and Mishra [26]. Reaction mixture contained 200 mM sodium phosphate buffer (pH 7.5), 0.1 M pyrogallol, distilled water and crude enzyme extract at 4°C. This was incubated for 5 min at 25°C after which the reaction was stopped by adding H_2SO_4. The amount of purpurogallin formed was determined by taking the absorbance at 420 nm. The enzyme activity was expressed in U g^{-1} FW.

8) Reactive Oxygen Species (ROS)

ROS production was measured as described by Able *et al*. [27]. The reaction mixture contained 50 mM K-phosphate buffer (pH7.8), 0.5 mm XTT and supernatant. The reaction of XTT was determined at 470 nm for 3 min. Corrections were made for the absorbance of chlorophyll. ROS production was calculated by using extinction coefficient of 2.163104 $M^{-1}cm^{-1}$.

III. RESULTS AND DISCUSSION

Deterioration is probably due to the field weathering or poor storage conditions, which is emerging now-a-days as one of the serious problems. Fifteen varieties specified above were used to compare different seed germination, seedling growth parameters and biophysical and biochemical parameters associated with antioxidative defense system.

A. Seed Germination and Seedling Growth Parameters

In the present investigation, different germination parameters were recorded to evaluate level of oxidative stress caused by field weathering/storage in 15 varieties of *E. sativa*.

A perusal of data set in table 2 shows that varieties exhibited different levels of germination percentage ranging from 30 – 100%. Varieties RTM – 1301 and RTM – 1356 shown 30% germination, in RTM – 1359 germination percentage was 40 %, whereas in RTM-314 and RTM – 1107 germination percentage was 50 and 60 %, respectively. Rest of the varieties exhibited 100% germination. Seeds of RTM- 1354 failed to germinate. Numerous workers have discovered that seed germination characteristics were affected by over storage period [28, 29, 30]. Reports are there that supports that the longer seeds storage period enhances the intensity of seeds aging [31,32,33,34]. Jatoi *et al*. [12] revealed that the rate of seed deterioration enhanced with the increased storage and also effected by storage temperature. As suggested by McDonald [28] and Hsu *et al*. [29], seeds that deteriorate rapidly by increasing the time of storage usually showed a noticeable decline in their ability to germinate. In addition, Mohammadi *et al*. [35] reported that seed deterioration results in diminished percentage of normal seedlings. Moreover, some researchers also demonstrated that the germination potential could be negatively affected by both natural and accelerated aging [36,37]. Decrease in SOG and COG was observed in varieties with oxidative stress (table 2). This decline in germination parameters could be correlated to physiological and biochemical changes during seed aging [38]. SOG was maximum in RTM – 673 and RTM - 1310 and lowest in variety RTM – 1301 whereas COG was maximum in RTM – 673 and RTM – 1310 and minimum in RTM – 1107. Rehman *et al*. [39] reported that per cent germination and speed of germination decreased significantly in *Acasia* seed by deterioration as compared to control. Reporters have also investigated the decrease in germination capacity, germination speed and seedling growth by natural aging in alfafa seeds [40,41]. Interestingly, highest MGT was recorded in RTM – 1356, (2.17) and lowest was observed in RTM – 673 and RTM – 1310 (0.2). Higher MGT was observed in varieties with low germination percentage and oxidative stress. Contrarily, Farahani and Chaichi [42] reported that

seed stored for one week in high temperature and relative humidity enhanced MGT in barley seed. Similarly, MGT increase in deteriorated seeds is also observed in soybean seed [43]. Khaje-Hoseini *et al*. [44] demonstrated that soybean deteriorated seeds took long time to germinate.

TABLE III
DATA REPRESENTING VARIOUS PARAMETERS OF SEED GERMINATION

Varieties	GP (%)	SOG	COG (%)	MGT
RTM-1212	100	4.83	39.58	0.26
RTM-1358	100	4.58	32.53	0.33
RTM - 603	100	4.83	39.58	0.26
RTM – 2002	100	4.58	32.53	0.33
RTM – 673	100	5	50	0.2
T-27	100	4.5	38.63	0.29
RTM - 1310	100	5	50	0.2
RTM – 1351	100	4.75	32.75	0.31
RTM – 1035	100	4.75	32.75	0.31
RTM – 1107	60	1.4	19.74	1.2
RTM-314	50	1.18	22.22	1.5
RTM – 1359	40	0.95	20	1.8
RTM – 1301	30	0.58	20.69	2.16
RTM – 1356	30	0.75	20.69	2.17

Minimum shoot and root length was observed in varieties having low percentage germination (table 3). Root and shoot length was significantly influenced by seed deterioration and oxidative stress. Kandil *et al*. [45] exhibited a significant effect of storage periods on the means of root length, shoot length in soybean cultivars. Their results revealed that shoot and root length decreases with an increase in storage period. Growth efficiency and seedling characteristics like shoot and root length decreased with aging [11]. Similar results were also found by Jatoi *et al*. [46], Jain *et al*. [47] and Munnujan *et al*. [48]. Interestingly, shoot dry matter was highest in RTM - 1310, (0.017 g) and lowest in RTM-1212, (0.008 g). Thus, sustained seed storage would enhance the metabolic activity of the seeds and therefore the reserve substance content and dry material weight of the seeds declined [49]. Oxidative stress may decrease the fresh and dry matter of the seedlings. Kandil *et al*. [45] observed that seedling fresh and dry weight was significantly affected due to the interaction between soybean cultivars and storage conditions. Seed vigor index is an indicator of rapid germination and speed of growth. According to table 3, it can be concluded that vigor index is significantly affected by seed deterioration. Deterioration is an indication of inability to reform functionally efficient membranes during rehydration of seeds resulting in lack of germination and loss of vigor [11]. Previous workers concluded that growth efficiency and seedling vigor gets influenced with aging [48,7]. The rapid loss of seedling vigor during aging might be used as a parameter to estimate the longevity of seeds during long term storage particularly in gene bank [12]. According to Walter *et al*. [50], the aging rate is strongly influenced by environmental and genetic factors such as seed moisture content, seed

quality and storage temperature. Rajjou and Debeaujon [51] exhibited that when seeds deteriorate during storage, they lose vigor, become more sensitive to stress during germination and eventually become incapable to germinate. The result of Kandil *et al.* [45] signifies the effect of storage periods on seedling vigor index.

A. Biophysical and biochemical Parameters

The electrolytic leakage (EL) indicates the membrane damage which increases significantly under stress conditions. EL was maximum in RTM – 1301 having, 30 % germination (table 4). Varieties with 100 % germination have low level of EL. Bhatia *et al*. [52] showed that increase in electrical conductivity in field weathered seeds of susceptible varieties was relatively higher when compared to unweathered seeds, suggesting that the possible reasons for the differences could be the genotypic response towards field weathering. Several biochemical investigations have demonstrated that lipid peroxidation and fat acidity (free fatty acid percentage) are the major reasons of seed deterioration, including cellular membrane disruption. According to Tilebeni and Golpayegani [13], an increase in free fatty acid content leads to a concurrent rise in electrical conductivity suggesting that membrane integrity had declined [53] . In the present investigation, RLWC level was low in the varieties having low germination percentage or having oxidative stress (table 4). RTM – 1359 (40 % GP) have minimum RLWC content. RLWC is considered to be an improved indicator of water status than water potential [54]. Drop in RLWC may occur due to reduction in stomatal conductance coupled with impaired water absorption [55].

TABLE IIIII
PARAMETERS OF SEEDLING GROWTH IN *E. SATIVA* VARIETIES

Varieties	SL (cm)	RL (cm)	FM (g)	DM (g)	VI
RTM-1212	3.43	4.03	0.16	0.01	746
RTM-1358	4.66	2.83	0.293	0.02	749
RTM – 603	4.13	4.56	0.242	0.013	869
RTM – 2002	3.26	4.6	0.193	0.017	786
RTM – 673	3.9	4.66	0.257	0.018	856
T-27	4.46	7.3	0.228	0.021	1176
RTM - 1310	3.43	3.6	0.306	0.023	703
RTM – 1351	4.03	5.23	0.209	0.016	926
RTM – 1035	3.53	6.23	0.229	0.018	976
RTM – 1107	2.76	1.86	0.169	0.013	277.2
RTM-314	1.9	1.92	0.184	0.012	191
RTM – 1359	2.45	2.6	0.199	0.013	202
RTM – 1301	1.89	1.9	0.19	0.012	113.7
RTM – 1356	2	1.63	0.184	0.013	108.9

TABLE IVV
ELECTROLYTE LEAKAGE (EL) AND RELATIVE LEAF WATER CONTENT (RLWC) IN ALL THE VARIETIES

Varieties	EL (%)	RLWC (%)
RTM-1212	37.81	85.84
RTM-1358	35.59	87.53
RTM – 603	33.59	89.68
RTM – 2002	39.51	84.46
RTM – 673	38.28	85.77
T-27	38.7	84.73
RTM - 1310	38.46	84.62
RTM – 1351	36.19	86.53
RTM – 1035	36.66	88.54
RTM – 1107	43.57	73.9
RTM-314	43.21	75.87
RTM – 1359	44.16	72.34
RTM – 1301	48.71	77.26
RTM – 1356	45.67	73.85

The increase in MDA content and electrical conductivity in field weathered seeds of susceptible varieties was relatively higher as compared to unweathered seeds [52]. The adverse effect of higher levels of free oxy radicals on lipid peroxidation and membrane integrity of field weathered seeds was also evident by Bhatia *et al*. [52]. Figure 2 shows the variable content of ascorbic acid in leaves and seeds of different varieties. In both leaves and seeds, maximum content was observed in RTM - 603 and minimum in RTM – 1301. The pattern of ascorbic acid showed a decline in the level with a prominent increase in the level of ROS. Present study was supported by the investigation made by Yadav *et al*. [56]. Bhatia *et al*. [52] also observed that in case of field weathered seed, maximum reduction in the level of ascorbic acid was observed within one week of weathering in JS 71-05 and two weeks of weathering in Punjab-1. This decline in ascorbic acid coincided with the maximum levels of oxy radicals in the seeds of these varieties. Proline acts as a compatible solute that regulates osmotic potential in the cytoplasm and participates in radical detoxification and enzyme protection [57]. Proline content was highest in leaves and seeds of RTM – 1107 and RTM-1354 respectively (figure 3).

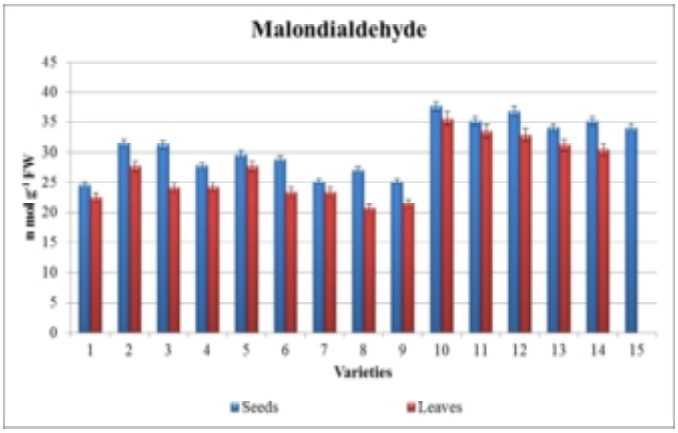

Fig. 1 Malondialdehyde (MDA) Content in Seeds and Leaves of *E. sativa* Varieties.

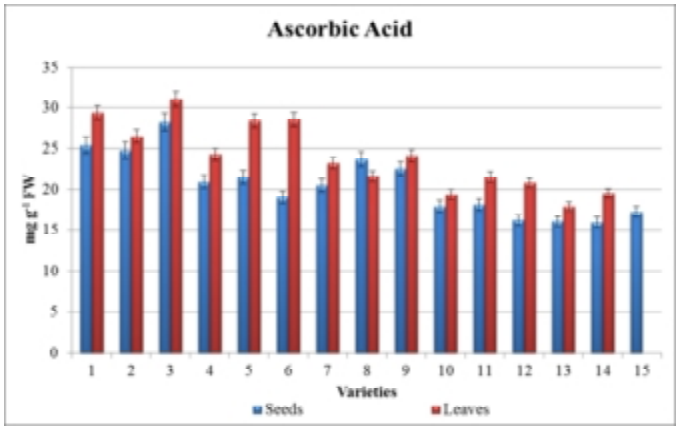

Fig. 2 Ascrobic Acid Content in Seeds and Leaves of *E. sativa* Varieties.

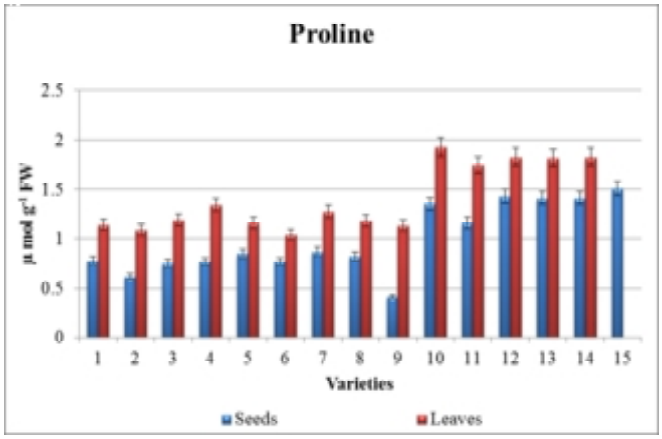

Fig. 3 Proline Content in Seeds and Leaves of Different Varieties

Peroxidases are constitutive enzymes of the cell wall of the intercellular spaces and vacuoles. Oxidative stress because of field weathering or storage reduces the activity of APX and POX in both seeds and leaves (figure 4 and 5). POX actively takes part in the oxidation of phenolic compounds [58]. The decline in peroxide-scavenging enzymes during weathering could decrease the cells ability to detoxify H_2O_2 and other peroxides. It is reported that the accelerated aging results in the reduced level of POX [59] and related signaling processes, and ultimately the seedling development [60]. Result may show that POX activity can be low in naturally aged dry seeds, but its activity may be influenced during germination. Cakmak *et al*. [41] observed that antioxidant enzyme (CAT, POX, and SOD) activity was low in the aged dry seeds. Their results showed that decrease in germination capability of the aged dry seeds of alfalfa was well linked with the increased levels of lipid peroxidation, and the decreased POX, CAT and SOD activities. CAT activity was high in varieties having 100% germination and low activity was observed in varieties with low per cent germination. The activity was high in leaves as compared to seeds in all the varieties. Cakmak *et al*. [41] reported that CAT activity was considerably low in the aged dry seeds of alfalfa as compared to non-aged ones. It has been proposed that the CAT activity usually decreases during accelerating seed aging [61,62]. In the studies made by Yadav *et al*. [63], a drastic reduction in the activity of CAT, APX and POD was observed in the seeds of soybean varieties, in response to weathering. The loss of activity increased in the seeds with increasing degree of weathering. In the present investigation we encountered that, the highest SOD activity was assayed in RTM – 1351 and the activity was lowest in RTM – 1107 (figure 7). SOD activity was maximum in leaves and minimum in seeds. Incidentally, the level of activity could be corroborated with percentage of germination. Bhatia *et al.* [52] has already reported that the SOD activity decreased continuously in field weathered seeds and this reduction in enzyme activity could lead to decrease in the capacity of the cells to

quench the free radicals produced and result in the higher seed deterioration. Some previous studies reported that the activity of SOD decrease during artificial aging of some aged seeds such as beech [64], cotton [62] and soybean [65]. Contrarily, Spychalla and Desborough [66] reported that there were not significant changes in free radical-scavenging enzymes such as SOD in older potato tubers.

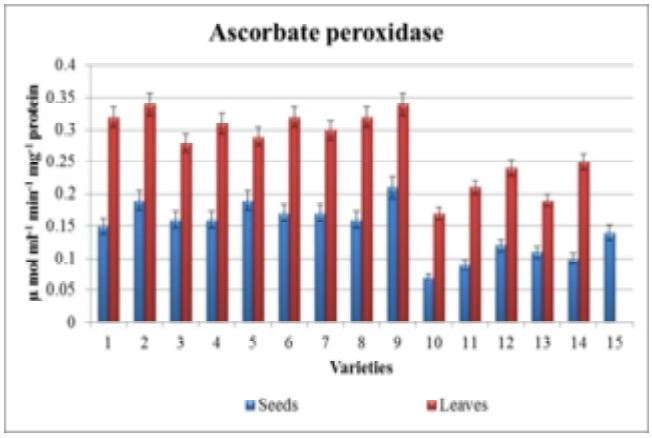

Fig. 4 Ascorbate Peroxidase Activity in All the Varieties (Seeds and Leaves).

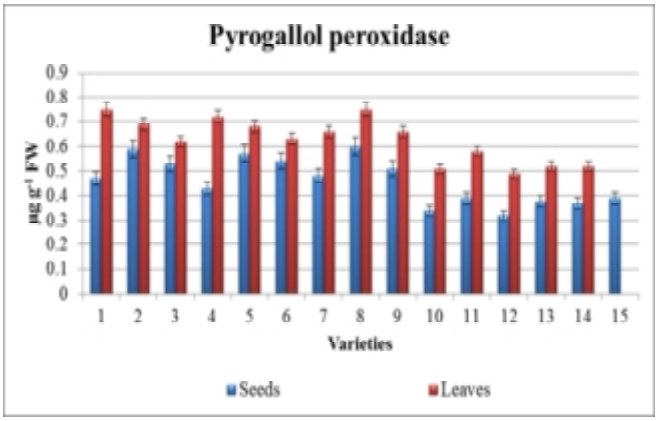

Fig. 5 Pyrogallol Peroxidase Activity in All the Varieties (Seeds and Leaves).

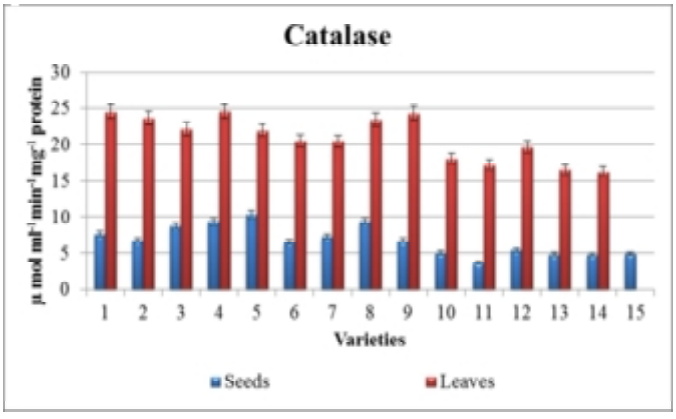

Fig. 6 Catalase Activity in Seeds and Leaves of All the Varieties.

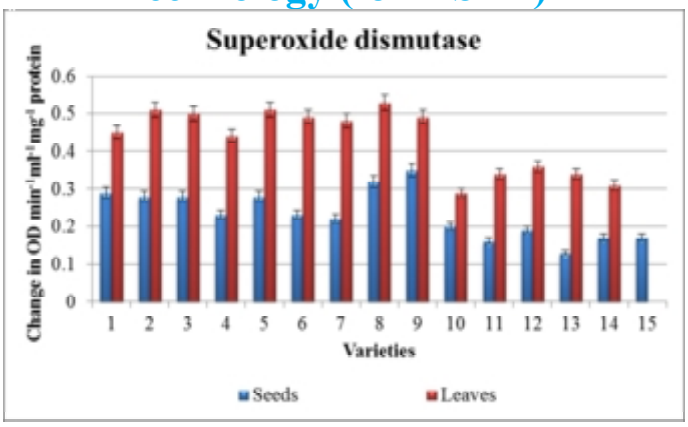

Fig. 7 Superoxide Dismutase Activity in Seeds and Leaves of All the Varieties.

Reactive oxygen species (ROS) is major cause of lipid peroxidation in cell membranes, generated not only in metabolism during stress and aging, but also in plant metabolism under normal conditions [67]. Excessive generation of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$) and the hydroxyl radicals ($^{\cdot}OH$) mostly in chloroplast and mitochondria cause rapid cell damage by triggering off a chain reaction [68]. Moreover, accumulation of ROS is potentially destructive for the growth and development [69]. The results obtained in the current study showed that accumulation of total ROS and lipid peroxides are associated with oxidative stress leading to a comparative study of ROS in seeds and leaves and revealing that the accumulation of ROS was higher in seeds (figure 8). Increased deterioration in seed quality and simultaneous increased free oxy radicals generation in weathered seeds compared to un-weathered seeds evidently revealed that the oxy radicals are involved in the deterioration of soybean seed quality during weathering [52]. ROS formation occurs naturally as a by-product of metabolism but their toxicity level was increased by environmental stresses [70], resulting in severe damage to cellular structures and ultimately, cell death [69]. Plant can detoxify ROS by up-regulation antioxidant enzymes, such as SOD, CAT, APX and POX as well as some non- enzymatic antioxidant compounds.

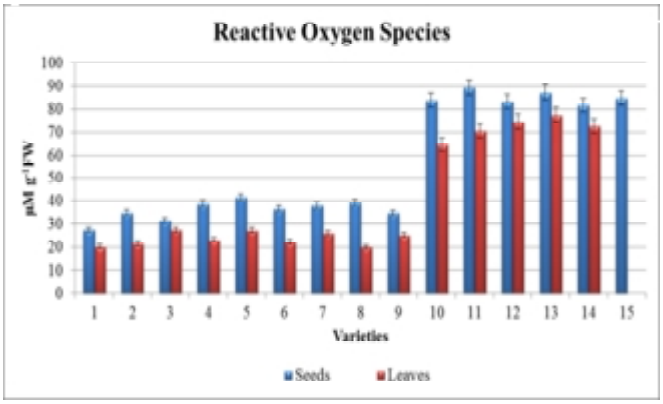

Fig. 8 Reactive Oxygen Species Level in Seeds and Leaves of Different Varieties.

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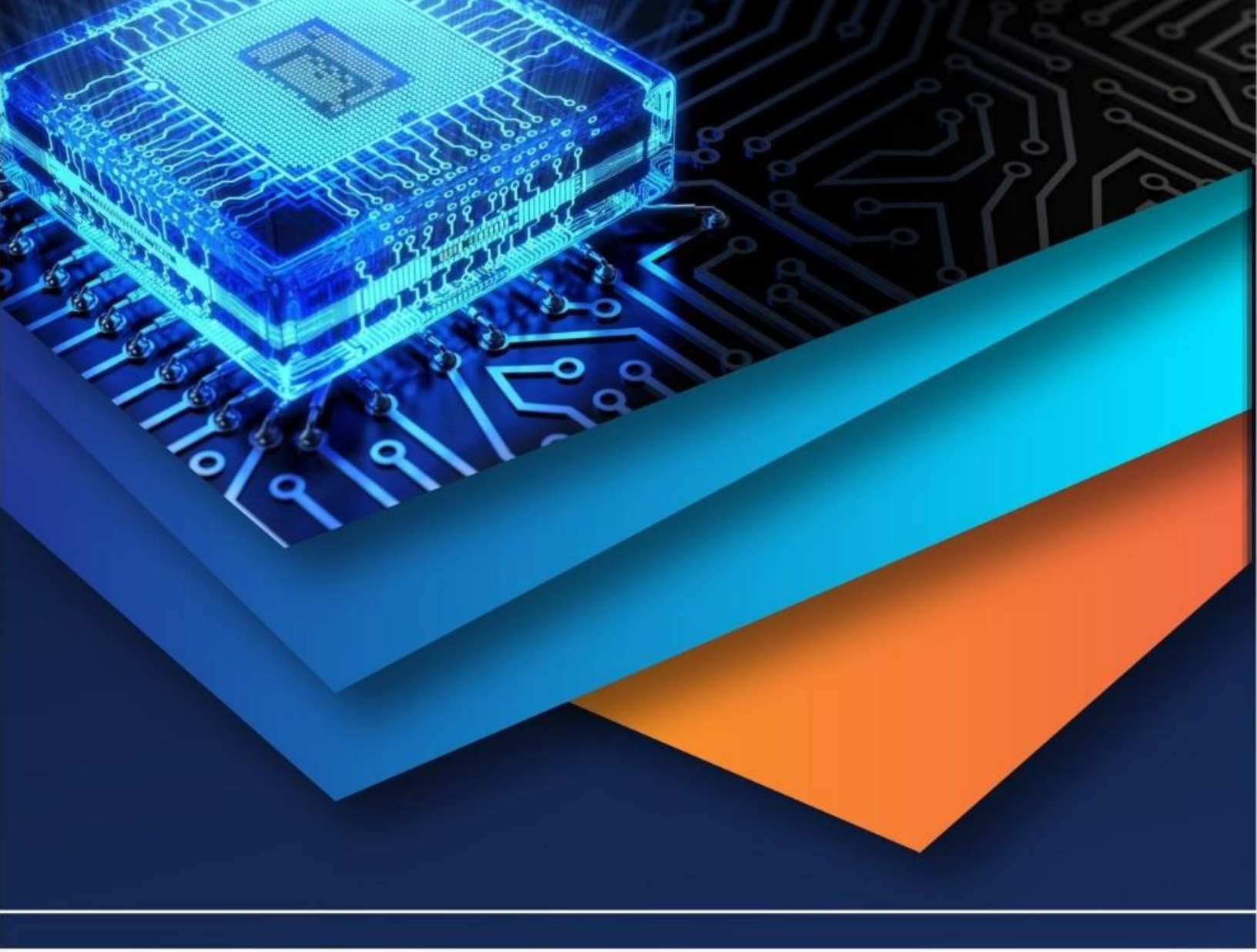

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