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Original article

Assessing aging impact on growth potential of Vitamin E primed soybean seeds via biochemical profiling

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ABSTRACT

Soybean [*Glycine max* (L.) Merr.] is a high value crop owing to its nutrient rich profile, consisting of some of the largest reserve of proteins and oils among all plant crops. High yielding soybean variety seeds are of great intrinsic value as part of strategies to gain larger yield outputs over the years. These seeds often tend to lose their viability and corresponding storage period. The study is primarily focused on understanding and estimating the impact of storage conditions and influence of biochemical changes that leads to deteriorating seed health. Vitamin E is an essential compound that provides shielding effect to plant seeds against environmental stress. For this purpose seed priming of vitamin E was performed with a concentration of $300 mgL^{-1}$ applied on to seeds. A total of seven promising cultivars were accessed for this; including Swat-20, Swat-84, NARC-2. Malakand, Rawal, Ajmeri and FaisalSoy. Results shows that all cultivars tend to lose their yield potential which is greatly in line with storage induced biochemical changes. Among the cultivars, Swat-84 and Swat-20 were resilient to an extent towards harmful storage stress impact. The present study has shown that application of vitamin E seed coating tend to enhance positive traits in stored seeds (including concentrations of CAT, SOD, TSS, etc.) in comparison to untreated seeds showing a healthy impact of the treatment on seed health under storage conditions. It is suggested that storing vitamin E treated seeds under optimum conditions as an effective method for attaining viable seeds after long terms storage. Findings of the present study can be used for future studies, assessment and designing of seed storage system in a manner to reduce negative impact on seed growth potential during long term storages.

1. Introduction

Soybean (*Glycine max* L. Merr.) is a major legume crop having a large stack in global food system. The soybean crop was first domesticated in the ancient China followed by the surrounding region of South-East Asia and was mainly consumed for its oil and protein-based products. Owing to its characteristically protein rich nutrition profile, the crop is a major food constituent for world population and particularly the people of Southeast Asia and is often termed as the "Cow of field" (Horvath, 1926, Hymowitz, 2008, Anderson et al., 2019). In modern day world, the soybean crop is cultivated almost all over the globe with its major production hubs located in the North-South America region (including the USA, Argentina, and Brazil) (Wilcox 2004, USDA-FAS 2018a, b). Just like in many other crops the seed of soybean also serve a dual purpose; as a source of food products (soymilk, tofu, soy sauce, etc.), as well as precursor for cultivation of next season crop. This production of crop can be viewed as a cyclic process where satisfying high-quality seed, due to their desired traits, are often stored for longer duration, utilized in various growing seasons, and maintained as part of reserve grains for longer durations. Occasionally these storage conditions can have some deleterious effects on the health of seed rendering it less viable. Seed health and vigour are of great importance as these factors have great impact over the

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Abbreviations: AA, Artificial Aging; NA, Natural Aging; NAE, Natural Aging Vitamin E treated; GP, Germination Percentage; MDA, Malondialdehyde; CAT, Catalase; TSS, Total Soluble sugars; RS, Reducing Sugars; SOD, Superoxide Dismutase; EC, Electrical Conductivity.

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productivity of the crops under different kind of soil and environmental conditions. Deterioration in seed health leads to negative impact on forecasted yields, and therefore is major topic of study in the field of plant sciences.

With global population currently standing at nearly 7.7 billion people and expected to rise up further to an approximate figure of nearly 9.8 billion in total by 2050, feeding this ever-increasing population is a major challenge. According to an estimate global food production must increase by over a 100% to meet up the global demands (Tillman et al., 2011). Meeting this global demand of food is not just about the quantity but also the quality of available food resources, as in existing times millions of children are deprived of healthy daily diet leading to various health complications eventually leading to death (Rice et al., 2000, Caulfield et al., 2004, Black et al., 2003). The issue of malnutrition in children is more prevalent in developing world and new challenges in form of climate change are meant to deteriorate this situation further risking food security for a major proportion of world population (Pelletier and Frongillo, 2003, Collins et al., 2006).

High performing cultivar seeds are stored for longer durations as their characteristically high yielding traits are of great significance, however, they seem to have lost their overall viability over time. This is primarily attributed to harsh storage conditions and result in major productivity losses (Shelar, 2008). Such conditions causes an onset of numerous bio-chemical, cytologic and pyshiological changes in seeds leading to deterioration of stored seeds (Jyoti and Malik, 2013). Furthermore, corresponding cultivars produce weak seedlings resulting in low productivity for previously high performing cultivars (Kapoor et al., 2010). Such outcomes triggers a high income loss phenomenon, threatening long term sustainable production for desired crops.

In order to deal with stress related degradation processes seed priming is often viewed as a pragmatic approach. Treating seeds with vitamins including Vitamin B, C, and E helps in mitigating negative impact of environmental stress by promoting transpiration rate, ATP synthesis, preventing DNA damage and other cellular deformities (Balestrazzi et al., 2011, Weitbrecht et al., 2011). The priming tends to trigger a metabolically high active state in seeds termed as “primed state” which helps to lessen stress related adverse effects (Savvides et al., 2016).

The objective of the present study is to understand series of biochemical event which leads to deterioration of seed health, and to devise a robust strategy for containing this deteriorating seed health. Events including the depression in levels of various antioxidant molecules (CAT, SOD, etc.), results in imbalance of antioxidant systems and corresponding accumulation of reactive oxygen species (ROS) cause a shift towards oxidative activities inside the seed. These biochemical changes in side the seed result in alteration of membrane permeability and chromosome aberration resulting in reduced viability of seed (Yin et al., 2014, water worth et al., 2015, Ebone et al., 2019).

2. Material and methods

2.1. Plant material

For the existing study, a total of seven different cultivars of soybean (Swat-20, Swat-84, NARC-2. Malakand, Rawal, Ajmeri and FaisalSoy) were used. The studied cultivars were obtained from Centre for advanced studies in agriculture and food security (CAS-AFS) at the University of Agriculture, Faisalabad, Pakistan. After harvest 1 kg seed for each of the cultivar was separated, and stored at 4 °C in Soybean Lab at CAS-AFS for further experimentation. Same amount of seeds was stored at room temperature.

2.2. Natural seed ageing and germination test

Seeds were separated in two batches; one with normal seeds and second including seeds treated with Vitamin E (seed priming). Level of moisture for these seeds was adjusted at about 11% (ISTA, 2014). Seeds were than packed in sterile boxes, sealed and stored at room temperature for further aging studies. For priming purpose 1 kg seed for each of the cultivar was surface sterilized using 3% NaOCl solution for 10 min. Afterwards, sterilized seeds were transferred in to Vitamin E solution (concentration 300mgL^{-1}) and soaked for 16 h. After that seeds were dried on sterile blotter paper and used for germination experimentation. Germination test was carried out for these seeds by wrapping in germination paper and placing in growth chamber. Standard of 4 mm radicle growth was selected to access the overall germination rate.

2.3. Artificial seed ageing and germination test

Moisture content for the seed was maintained at around 11% as per the guidelines from ISTA (2014). Seeds were collected in sterile plastic box and exposed to artificial ageing experiment conditions at 100% relative humidity (RH) and 40 °C under three different time sets (40 h, 60 h and 80 h). Hundred seeds per box were taken for the experiment and remaining were stored at 4 °C. For germination test hundred seeds from each genotype were wrapped in wet germination paper, placed into growth chambers at 25 °C for two weeks and the remaining seeds were used for biochemical analyses (Brazil-Ministry of Agriculture, 2009,). For measuring the germination rate a radicle length of 4 mm was set as standard germination criteria.

2.4. Biochemical analyses

For biochemical analysis 5 seed per genotype were taken and macerated in 4 mL potassium phosphate buffer 100 mM (pH 7.0).

2.5. Malondialdehyde (MDA) measurement

Malondialdehyde (MDA) concentration analysis was performed following protocol explained by Jiang et al., (2018), final concentration of MDA was measured by an absorption of 530 nm with an absorbance coefficient of 155 mM^{-1} cm$^{-1.}$

2.6. Sugars content

Concentration analysis for total soluble sugar (TSS) and later for the reducing sugar (RS) content were carried out, for the TSS content estimations phenol-acidic assay was carried out as explained by DuBois et al., (1956), in this a clear aqueous solution of sample was added in test tube followed by addition of phenol and sulphuric acid into the tube. Reaction between sugars and phenol gave yellowish-orange colour, resultant pellet was treated with perchloric acid and corresponding aliquots were used for determination of sugar contents (mg g^{-1}). Concentration of reducing sugar (RS) was estimated following the protocol given by Miller (1959).

2.7. Catalase activity

Catalase (CAT) activity was estimated following protocol method given by Havir and McHale (1987), for this crude sample extract was added into potassium phosphate buffer, containing hydrogen peroxide in it, at 30 °C temperature. Absorbance for the reaction was measured at 240 nm for 5 mins. and CAT activity was calculated and expressed in μmol min^{-1} mg protein^{-1}.

2.8. Superoxide Dismutase (SOD) activity

SOD activity was estimated following protocol given by Giannopolotis and Ries (1977), crude sample extract was added into potassium phosphate buffer and a mixture of methionine, riboflavin and nitro-blue tetrazolium chloride (NBT) was added in it at 25 °C temperature. Activity for SOD was measured at 540 nm absorbance.

2.9. Electrical conductivity (EC)

EC was estimated following protocol given by Seyyedi et al., (2018), 40 pre-weighed seeds were placed in sterile water at 25 °C for 24 h interval. EC meter was used to measure the value of electric conductivity and further expressed in $\mu S\ cm^{-1}\ g^{-1}$ according to the equation given by Hajiabbasi et al., (2015).

EC ($\mu S\ cm^{-1}\ g^{-1}$) = EC for each sample ($\mu S\ cm^{-1}$) / weight of seed sample (g).

2.10. Statistical analysis

Data was collected for each of the trait/parameter and subjected to statistical analysis. Analysis of variance (ANOVA) for each of the trait was carried out, keeping in view the cultivar and time, in complete randomized design (CRD), followed by LSD test with a 5% probability ($p < 0.05$) and correlation estimation for factors under study (Table 4.1).

3. Results

3.1. Germination response under artificial ageing

Germination for each of the cultivars was estimated at zero exposure and further leading to exposure of extreme conditions at three different time periods (40 h, 60 h and 80 h interval). At zero deterioration levels, all genotypes expressed high rate of germinations with each of them giving > 80% germination rate, i.e. Swat-20 gave 87% germination, swat-84 92%, NARC-2 87%, Malakand 90%, Rawal 83%, Ajmeri 92%, and FaisalSoy gave a 92% germination rate. After first round of artificial ageing at the end of 40 h duration (at 40 °C and 100% RH) significant reduction in germination rates for all cultivars were recorded with>30% reduction in germination observed for Swat-20, while other cultivars (Swat-84, NARC-2, Malakand, Rawal, Ajmeri and FaisalSoy) experienced>40% reduction in seed germination rate. After second stage ageing with 60 h exposure to adverse conditions (at 40 °C and 100% RH) further decrease in germination rates were recorded with Swat-20 and Swat-84 showing significant decrease with 18% and 19% respectively, followed by other cultivars with each of them giving around 15% reductions in germination. Further 15–20% reduction in germination rate was recorded for all cultivars after 80 h of artificial ageing (under 40 °C and 100% RH) (Fig. 4.1A).

3.2. Germination response under natural ageing

Germination for each of the cultivar was estimated under natural ageing from both batches; normal stored seeds and vitamin E treated stored seeds. Germination levels for all non-treated cultivars showed significant losses with more than two third reduction in growth percentages recorded for all cultivars. In comparison seeds treated with Vitamin E gave slightly better results with slightly>50% reduction in growth percentages recorded for all cultivars. Overall a general reducing trend in germination percentages was recorded for all cultivars under both conditions (Fig. 4.1B and C).

3.3. Biochemical analysis

A detailed biochemical analysis for key chemical compounds presents in seed was carried out. The prime objective for this was to estimate biochemical activity inside seed and its impact on germinations with respect to artificial ageing conditions.

3.4. Malondialdehyde (MDA)

In artificial aging studies during initial stage of the experiment concentration for the MDA was relatively lower in all cultivars ranging an average 0.047 to 0.060 nmole g^{-1}. Artificial aging resulted into accumulation of MDA molecules in seed indicating an increase in concentrations in response to ageing stress. After

Table 4.1
Pearson's Correlation for multiple biochemical factors and different seed aging trials for each particular Cultivar.

Traits	Cultivars						
	Swat-20	Swat-84	NARC-2	Malakand	Rawal	Ajmeri	FaisalSoy
GP-AA	−0.99885	−0.99918	−0.99798	−0.99156	−0.997043	−0.99397	−0.99615
GP-NA	−1	−1	−1	−1	−1	−1	−1
GP-NAE	−1	−1	−1	−1	−1	−1	−1
MDA-AA	0.999836	0.993657	0.993607	0.999571	0.9893224	0.986799	0.99133
MDA-NA	1	1	1	1	1	1	1
MDA-NAE	1	1	1	1	1	1	1
CAT-AA	−0.99784	−0.99819	−0.99747	−0.998	−0.998614	−0.99791	−0.99869
CAT-NA	−1	−1	−1	−1	−1	−1	−1
CAT-NAE	−1	−1	−1	−1	−1	−1	−1
TSS-AA	−0.9971	−0.99233	−0.99302	−0.9954	−0.969746	−0.99027	−0.98047
TSS-NA	−1	−1	−1	−1	−1	−1	−1
TSS-NAE	−1	−1	−1	−1	−1	−1	−1
RS-AA	0.995069	0.969746	0.990296	0.980504	0.9920521	0.986914	0.999915
RS-NA	1	1	1	1	1	1	1
RS-NAE	1	1	1	1	1	1	1
SOD-AA	−0.99968	−0.99621	−0.99927	−0.9946	−0.995206	−0.9966	−0.98779
SOD-NA	−1	−1	−1	−1	−1	−1	−1
SOD-NAE	−1	−1	−1	−1	−1	−1	−1
EC-AA	0.958874	0.922707	0.940097	0.941989	0.9470507	0.943138	0.939298
EC-NA	1	1	1	1	1	1	1
EC-NAE	1	1	1	1	1	1	1

AA = Artificial Aging, NA = Natural Aging, NAE = Natural Aging Vitamin E treated, GP = Germination Percentage, MDA = Malondialdehyde, CAT = Catalase, TSS = Total Soluble sugars, RS = Reducing Sugars, SOD = Superoxide Dismutase, EC = Electrical Conductivity

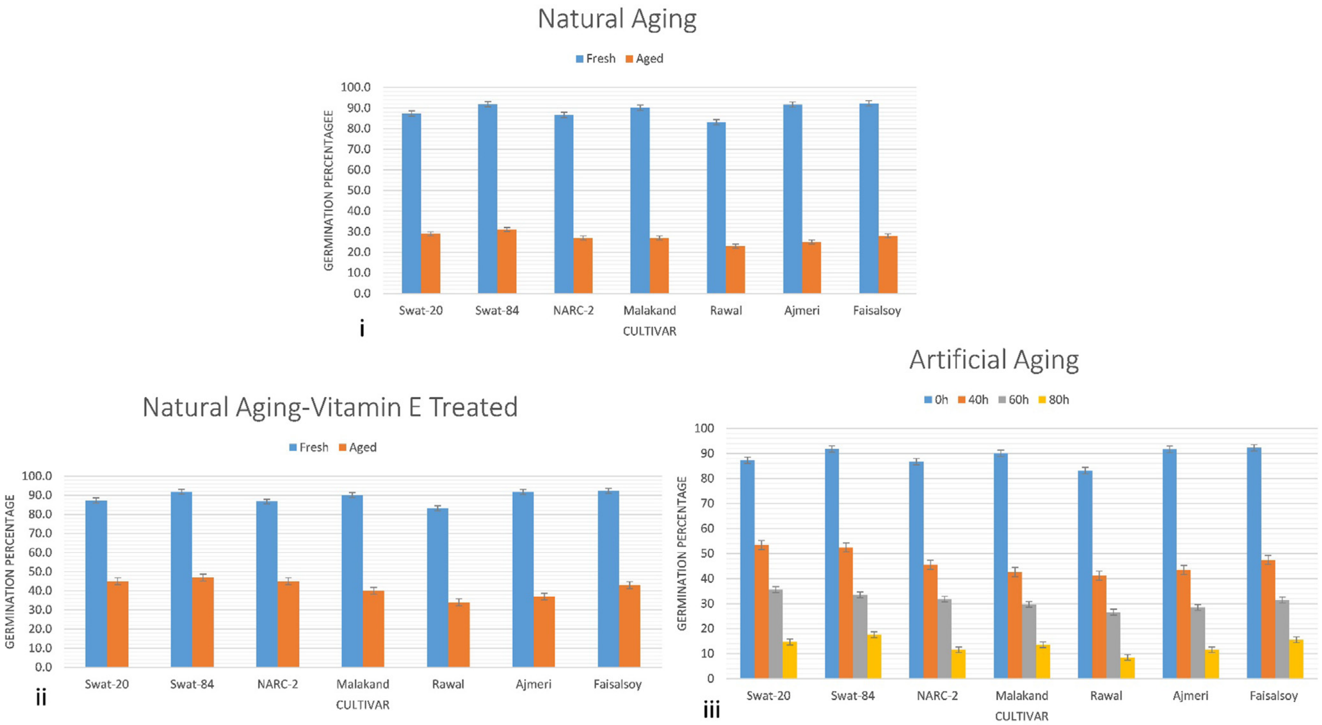

Fig. 4.1. Recorded impact of storage conditions on percentage germination for cultivars under (i) Natural aging conditions (ii) Natural aging with vitamin E treated and (iii) under Artificial aging.

40 h of ageing exposure, MDA concentrations were found in range of 0.29 to 0.51 nmole g^{-1} (indicating an overall increase by a factor of 0.24 to 0.45 nmole g^{-1}). Further increase in MDA concentrations were recorded, after second ageing 60 h, ranging from 0.41 to 0.66 nmole g^{-1} (increase by a factor of 0.11 to 0.22 nmole g^{-1}). Third ageing caused further alleviation in MDA levels with recorded concentration ranging from 0.61 to 0.81 nmole g^{-1}(increase by a factor of 0.05 to 0.14 nmole g^{-1}) (Fig. 4.2 A). Overall effect of ageing was evident on levels of MDA with gradual increase in its concentrations observed throughout the study. For natural aging process both the treated (with vitamin E) and untreated seeds exhibited nearly identical results at initial stages ranging from 0.047 to 0.060 nmole g^{-1} and the resulting values were very close to the one expressed in artificial aging experiment as well.

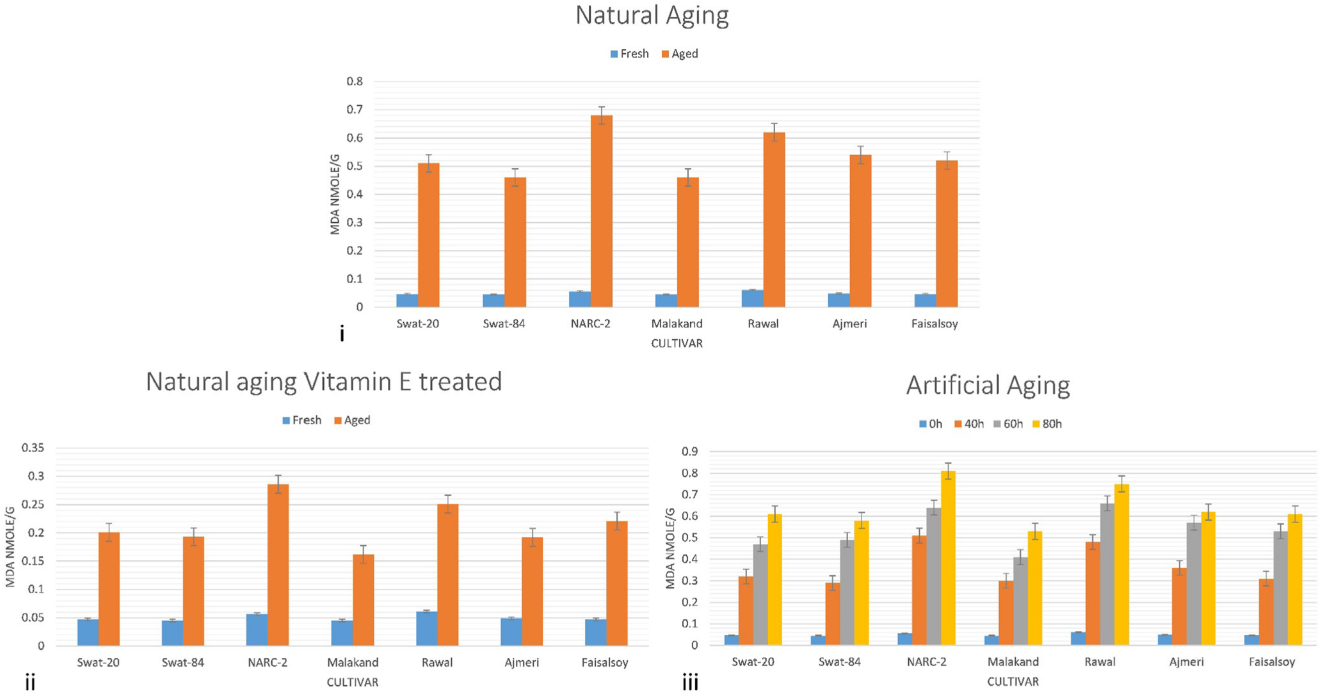

Fig. 4.2. Recorded impact of storage conditions on MDA concentrations in each cultivar (i) under natural aging (ii) under natural aging Vitamin E treated (iii) under artificial aging.

This was a clear indication of different cultivars giving significant responses under the influence of storage conditions leading to wide range of results in post storage utilization of seeds. After natural aging test treated seeds showed MDA concentrations ranging from 0.20 to 0.28 nmole g^{-1} , whereas untreated seeds presented significantly higher values ranging from 0.46 to 0.62 nmole g^{-1} (Fig. 4.2 B and C).

3.5. Sugar contents

Levels of total soluble sugars (TSS) were observed for normal seeds and those exposed to artificial ageing. Initially each of the cultivar type exhibited fair level of TSS contents with Swat-20, Malakand and Swat-84 exhibiting highest levels among all with a concentration of 4.1, 4.2 and 4.3 mg g^{-1} respectively, followed by fair level of TSS in other cultivars (NARC-2, Rawal, Ajmeri, FaisalSoy) ranging from 2.9 to 3.6 mg g^{-1}. After initial exposure to adverse ageing conditions for 40 h each cultivar significantly lost TSS content; with Rawal showing highest levels of content loss 0.8 mg g^{-1} followed by Swat-20 and Swat-84 with 0.6 mg g^{-1} loss, Malakand 0.5 mg g^{-1}, NARC-2 and Ajmeri 0.4 mg g^{-1}, and Faisal soy 0.3 mg g^{-1}. Second period of exposure to ageing conditions for 60 h resulted in further reduction of TSS content ranging from 0.2 to 0.5 mg g^{-1}. Final ageing exposure for 80 h caused further reduction in TSS content by a factor of 0.1 to 0.3 mg g^{-1}, although this reduction was lowest among all ageing stages still a gradual increase indicates negative impact of ageing on seed TSS content (Fig. 4.3 A). Level for reducing sugars (RS) were also estimated with reference to ageing of seeds. On each stage (40 h, 60 h and 80 h) concentration of RS was estimated for all cultivars with final levels (after 80 h) being twice as compared to the initial levels (Fig. 4.4A).

For natural aging studies, seed TSS contents were estimated before and after aging process (for both treated and untreated seeds). At initial stage, seeds exhibited similar results in comparison to artificial aging studies ranging from 2.9 to 4.1 mg g^{-1}, after aging process all cultivars treated with Vitamin E gave better results in comparison to their untreated counterparts. Cultivars Swat-20 and Swat-84 exhibited best results in both studies whereas Rawal showed worst response under both scenarios (Fig. 4.3 B and C).

Reducing sugars (RS) content concentrations were also estimated for natural aging experimentation with cultivars Swat-20 and Swat-84 presenting best response (for both treated and untreated) and cultivars Rawal and Ajmeri giving least favourable results (Fig. 4.4 B and C).

3.6. Catalase (CAT)

Catalase is an important antioxidant molecule and act as major health component for seed. In this reference estimation of CAT activity is of significant importance for accurate assessment of ageing affect on seed health. A gradual decreasing trend for CAT activity was evident in the present study, with initial ageing exposure (at 40 °C and 100% RH for 40 h) resulting in > 40% loss in CAT activity. Second round of ageing exposure (at 40 °C and 100% RH for 60 h) produced a further negative impact on CAT activity with five cultivars; Swat-20, Swat-84, Malakand, Ajmeri and FaisalSoy recording further losses of > 40%, whereas two cultivars NARC-2 and Rawal exhibited > 50% loss in CAT activity. Third round of ageing exposure (at 40 °C and 100% RH for 80 h) gave more diverse results than before with each of the cultivars showing different level of resilience to ageing after higher exposure of harsh conditions; Rawal cultivar showed a loss of about 78% in CAT activity whereas NARC gave around 70% further losses, Malakand, FaisalSoy and Ajmeri recorded about 60% fall in CAT activity, while Swat-20 and Swat-84 demonstrated about 50% further losses (Fig. 4.5 A).

CAT activity under natural aging was estimated as well, for both Vitamin E treated seeds and untreated seeds; treated seeds showed better response with nearly 40% reduction in CAT activity reported for all cultivars after aging, whereas for untreated seeds > 50% reductions in seed CAT activity were recorded. Cultivars Swat-20 and Swat-84 gave best response whereas Rawal was least effective in responding to natural aging stress under both scenarios (Fig. 4.5 B and C).

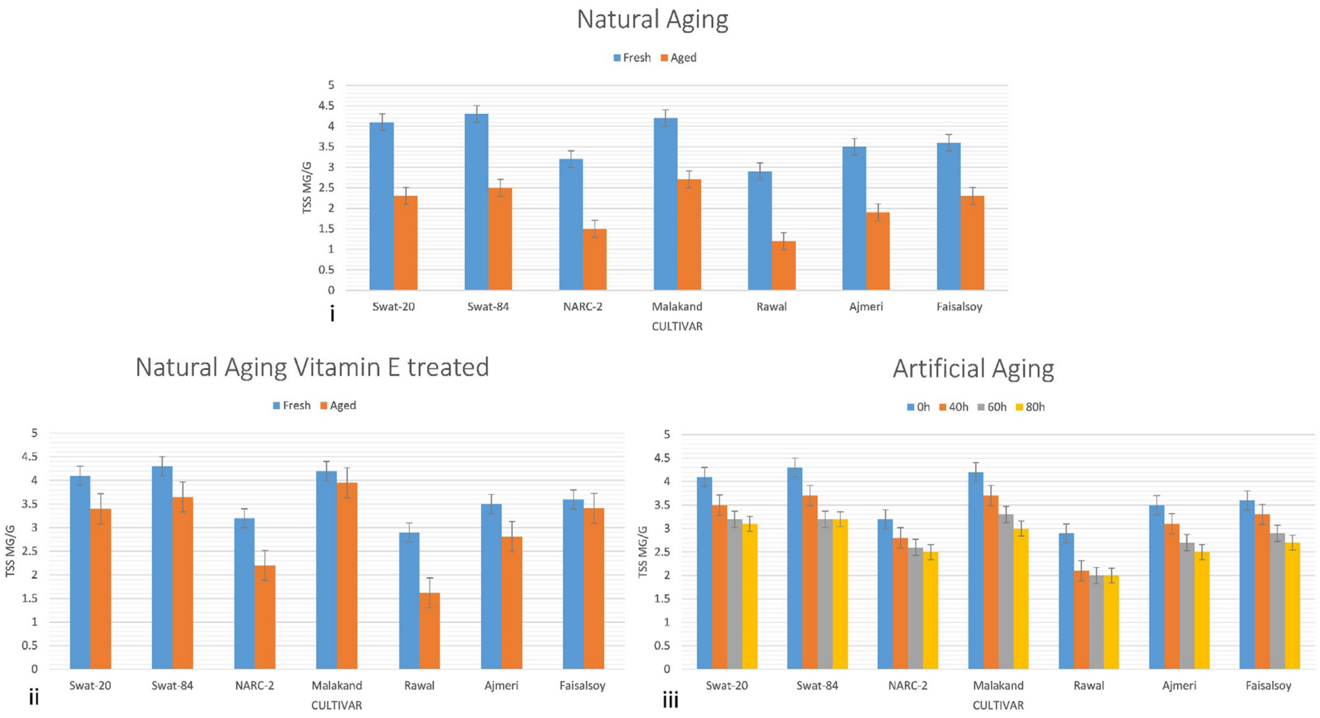

Fig. 4.3. Recorded impact of storage conditions on TSS concentrations in each cultivar (i) under natural aging (ii) under natural aging Vitamin E treated (iii) under artificial aging conditions.

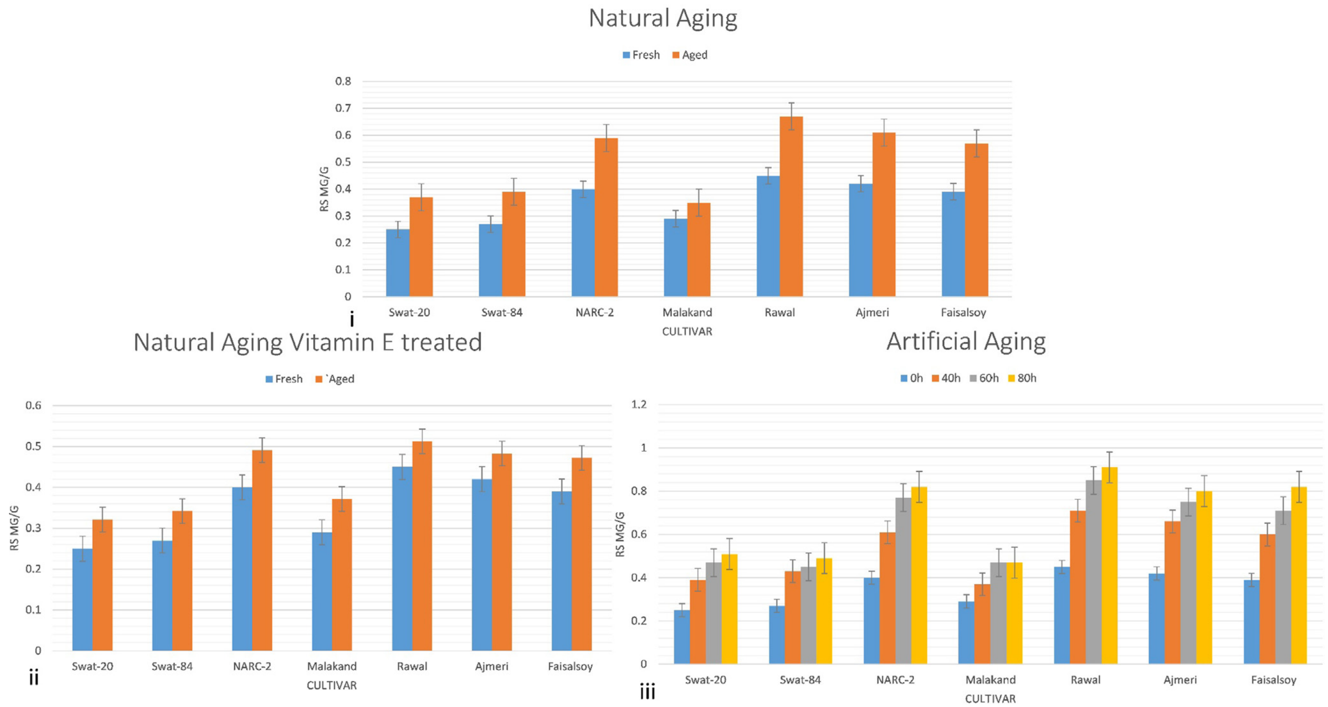

Fig. 4.4. Recorded impact of storage conditions on RS concentrations in each cultivar (i) under natural aging (ii) under natural aging Vitamin E treated (iii) under artificial aging conditions.

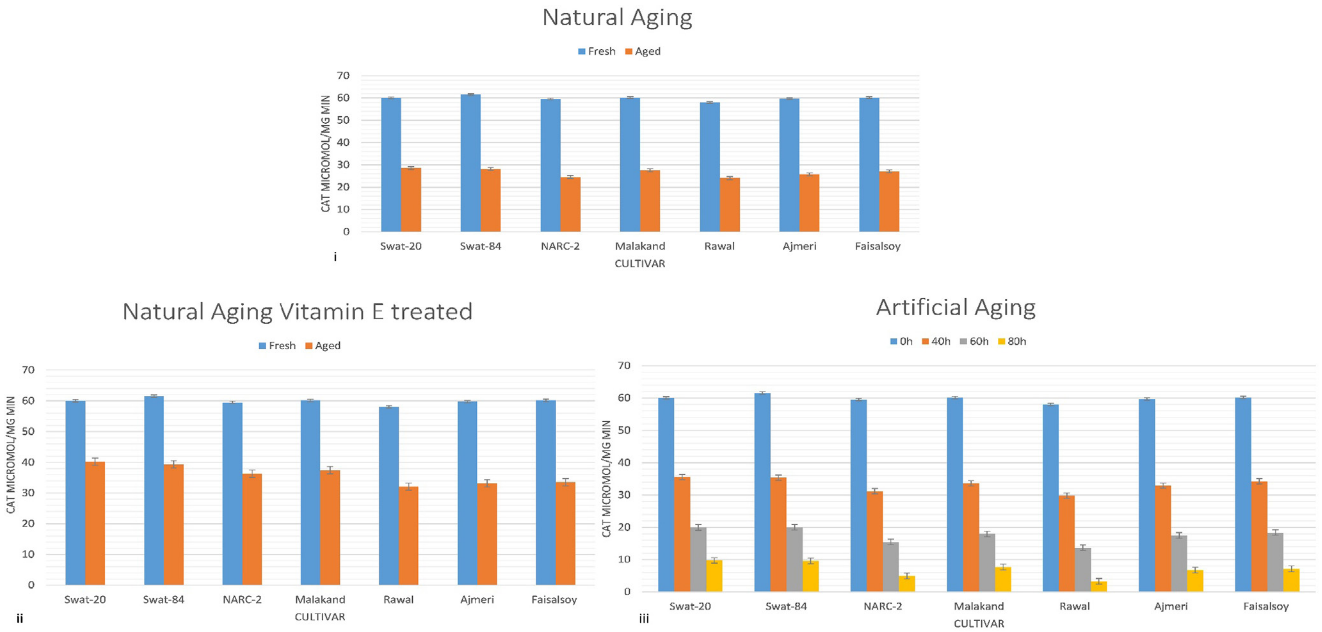

Fig. 4.5. Recorded impact of storage conditions on RS concentrations in each cultivar (i) under natural aging (ii) under natural aging Vitamin E treated (iii) under artificial aging conditions.

3.7. Superoxide dismutase (SOD)

SOD is another major antioxidant molecule present in seeds, its concentrations have been observed for the entire study; before, during and after the ageing process and has shown a significant impact of ageing on each cultivar. FaisalSoy exhibited highest degree of SOD deterioration as the concentration for SOD activity reduced from 47.88 μmol min^{-1} mg protein^{-1} at initial stage, down to 0.5 μmol min^{-1} mg protein^{-1} after final ageing (at 40 °C and 100% RH for 80 h). Other cultivars including Swat-20, NARC-2, Rawal and Ajmeri also gave significantly lower numbers for final concentrations of SOD activity with an average 3.55, 7, 1.3 and 2.3 μmol min^{-1} mg protein^{-1} SOD concentrations after final ageing. Two cultivars expressed a higher degree of sustainability for final concentration of SOD activity, with an average of 9.1 and 16.4 μmol min^{-1} mg protein^{-1} final concentrations (Fig. 4.6 A).

SOD activity for natural aging also exhibited a gradual decreasing trend with Vitamin E treated seeds showing nearly one third decrease in SOD concentrations and for untreated seeds the activity was reduced to more than half of initial values (Fig. 4.6 B and C). Among all both Swat cultivars (20 and 84) showed certain degree of effectiveness against harsh storage conditions.

3.8. Electrical conductivity (EC)

Electrical conductivity was estimated for seeds of each genotype as degradation tends to damage the membrane structures within the seed resulting in electrolyte leakages. Value of EC for each of the given cultivar exhibited over four times increase from initial stage to the final ageing. Rawal cultivar showed the highest values with an increase from an initial value of 9.4 to 43.6 μS cm^{-1}-g^{-1} after final exposure of ageing. NARC-2 showed second highest increase in EC with 30.8 μS cm^{-1} g^{-1} increase between initial stage values and after final ageing, followed by Ajmeri and FaisalSoy with an increase of 28 and 29 μS cm^{-1} g^{-1} respectively. Swat cultivars (Swat-20 and Swat-84) exhibited least increase in EC value with estimated nearly 22 μS cm^{-1} g^{-1} increase in value (Fig. 4.7 A).

EC values were also estimated for natural aging process in line with the concept that all type of seed aging tends to cause damage at cellular level and that the increase in EC activity is an indicator of seed deterioration owing to cellular content leakage. For natural aging conditions of seeds EC values for both treated and untreated batch were estimated with almost all exhibiting gradual increase at pre and post aging estimations. For treated batch of seeds all cultivars showed nearly 30–40% increase in EC activity with an exception of one cultivar (Malakand) which showed nearly 45% increase in EC activity (Fig. 4.7 B). For untreated batch all cultivars showed high increase in EC value with reported increase of 50–60% evident in all (Fig. 4.7 C).

3.9. Statistical analysis

Recorded data was subjected to statistical analysis using IBM SPSS Statistics 22, Pearson's correlation was estimated for factors under study with overall wide range of response being recorded from all factors with a range of 1 to −1 (Table 4.1). Factors giving positive response tend to have a positive correlation, in the current study growth limiting and seed health deteriorating factors including; Malondialdehyde (MDA), Reducing Sugars (RS) and electrical conductivity (EC) showed high degree of positive correlation response ranging from 0.93 to 1. Meanwhile factors recording a negative response tend to have negative correlation, factors including Catalase (CAT), total soluble sugars (TSS), Superoxide Dismutase (SOD), all showed negative responses ranging from −0.98 to −1. The final analysis of correlation for germination percentage and seed aging showed negative response of −0.99 to −1, indicating a high degree of negative correlation between the two. This shows overall aging has severe impact on growth and germination of seeds and can ultimately bring down total yield outputs of crops. Correlation for all factors is recorded in Table 4.1, Fig. 4.8.

4. Discussion

Soybean (*Glycine max L. Merr.*) is a key crop for global food system; as it has a rich nutrient profile and is used as human food and livestock feed (Kumari et al., 2015). The objective of the current study was to understand biochemical trait changes in seed due to rapid ageing and its corresponding impact on seed viability and germination. The study was inspired from hypothesis presented by Ebone et al., (2019), explaining about rapid deterioration of seed health under ageing stress. Concise results for the present study have shown a significant impact of ageing process and storage conditions on seed health. Previous studies have revealed a sense of correlation between seed ageing and imbalance in biochemical profiles for seeds, leading to low germinations and growth for the cultivars (Clerkx et al., 2004, Fessel et al., 2006, Açıkgöz et al., 2013).

In the current study, a total of seven high performing genotypes were utilized and impact of ageing on their individual biochemical profile was observed. Firstly, CAT concentrations were observed as

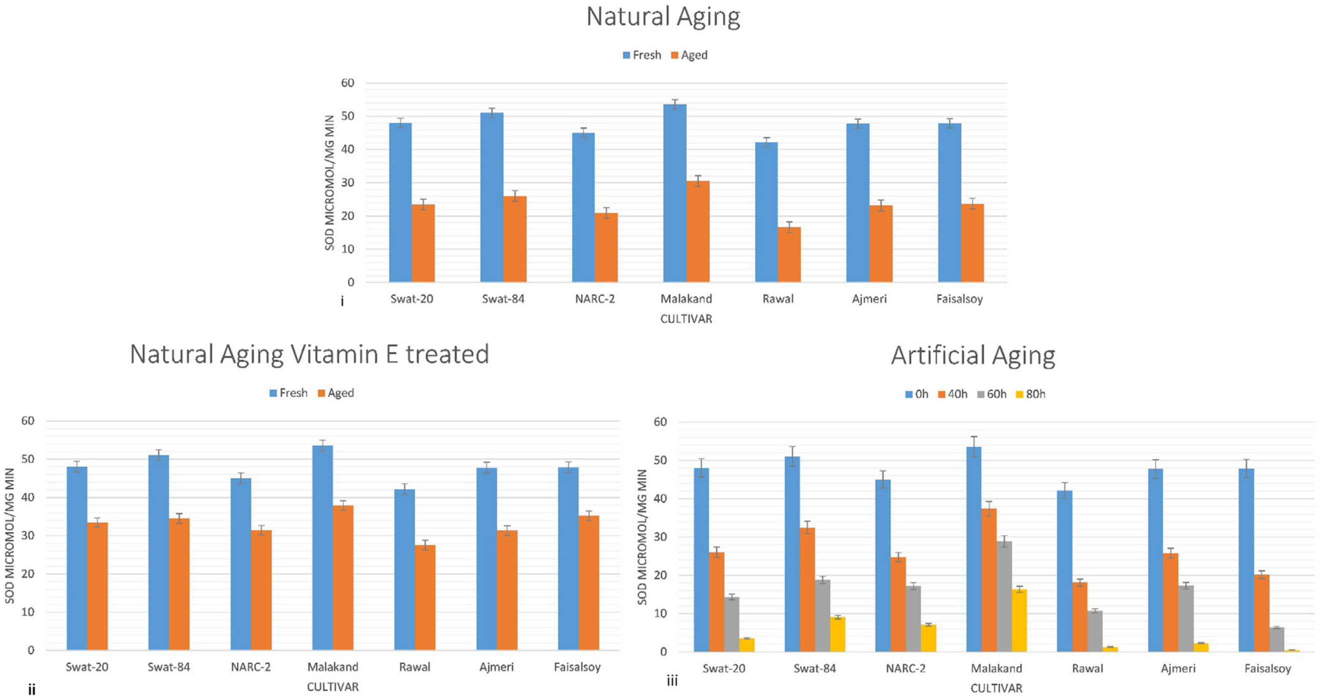

Fig. 4.6. Recorded impact of storage conditions on SOD concentrations in each cultivar (i) under natural aging (ii) under natural aging Vitamin E treated (iii) under artificial aging conditions.

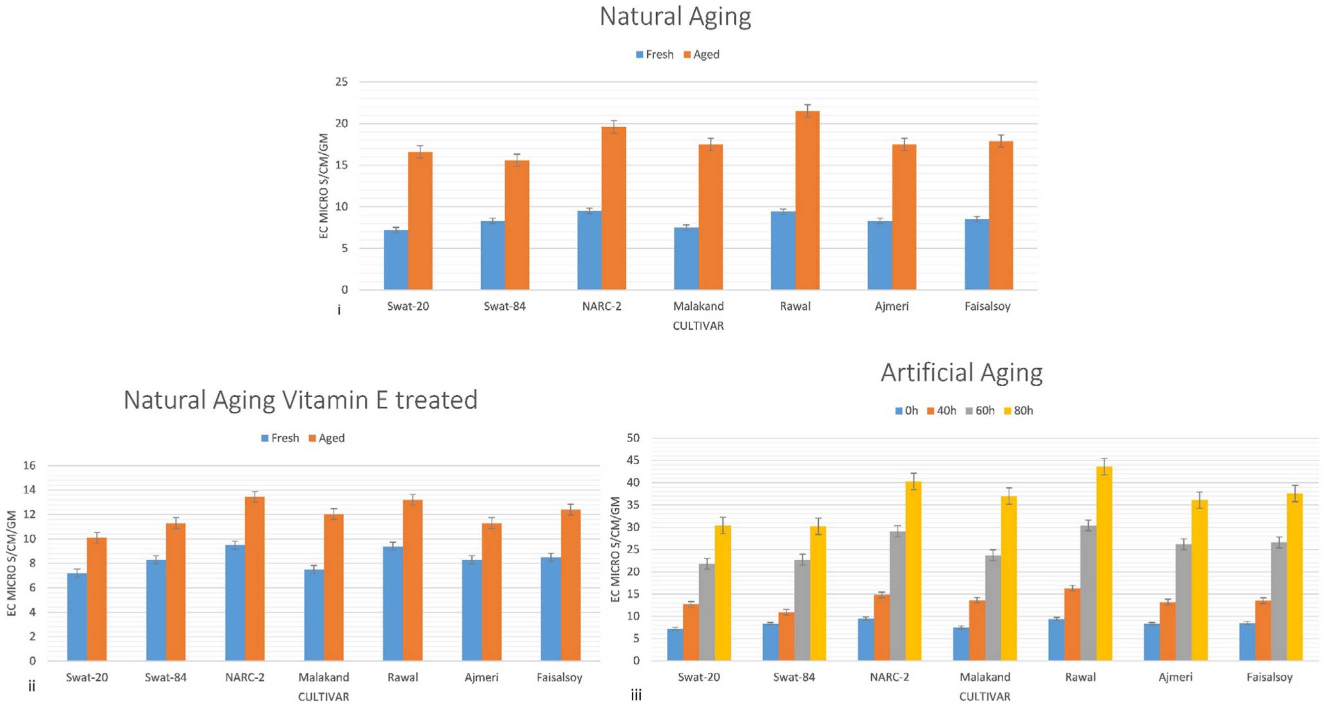

Fig. 4.7. Recorded influence of storage conditions on EC values for seeds of each cultivar (i) under natural aging (ii) under natural aging Vitamin E treated (iii) under artificial aging conditions.

it's an important enzyme for seed survival. It plays a key role in conversion of harmful hydrogen peroxide into water and normal oxygen under certain oxidative stress conditions (Chelikani et al., 2004, Bakalova et al., 2004). Among all the cultivars significant reduction of CAT activity was evident from given results for each of the seed type; gradual decrease in CAT concentrations was in line with reduced germination for all germplasms. It was observed that under moderate exposure to stress conditions the trend for deteriorating conditions was constant among all cultivars, however, with prolonged exposure a diverse response was recorded with 2 cultivars showing more 70% reduction, 3 exhibiting around 60% reduction response and 2 exhibited around 50% reduction in CAT content. Concentrations for SOD were also estimated, which is another important anti-oxidant compound responsible for shielding numerous cellular components from reactive oxygen species (ROS). Significant reduction in SOD content was recorded in all cultivars under rapid ageing stress indicating a deteriorating trend which hampers the growth and germination of seeds.

Depressions in antioxidant levels give rise to an imbalance situation that tends to favour cellular deterioration process due to accumulation of reactive oxygen species (ROS). Accumulating ROS causes lipid per-oxidation of cellular components, consumption of reserve materials in seed and production of malondialdehyde (MDA) content. These oxidative damages lead to produces certain geno-toxic effects on seed; causing damage to the DNA structure, reduction in nascent mRNA content and harming the ability to maintain seed embryo (Gidrol et al., 1988, Ayala et al., 2014, Ebone et al., 2019). MDA analysis for all seeds under ageing stress test gave a positive relation between deteriorating seed health and gradual increase in MDA concentrations, which is another key indicator of ageing effect as MDA is final product for lipid per-oxidation process indicating higher degree of degradation for cellular contents under ROS stress due to ageing process (Chen et al., 2013, Ayala et al., 2014).

Cellular disruption also leads to leakage of electrolyte contents resulting in sudden elevation of EC conductivity which was evident from our recorded results throughout the ageing experiment. In the present study, all cultivars under stress conditions exhibited increased EC conductivity indicating loss of essential nutrients and electrolytes inside seed leading to deprivation of food supplies having an ultimate negative impact on seed viability (Seyyedi et al., 2018). Depression in food supplies alongside physio-chemical changes in mitochondrial membrane leads to energy shutdown in seed leading to damage of embryo health, making it less viable for further growth (Daum et al., 2013).

Evident from our ongoing study, and supported by previous reports (Wilson, 1986, Basra et al., 1994; Sattler et al., 2004), is the fact that extended storage of seeds result in gradual loss of viability in seeds. In our current investigation it has been observed that seed priming with vitamin E helps greatly in ameliorating negative effects of seed aging. Vitamin E compounds are also naturally produced in plants, with chloroplast and plastoglobuli being its main centre of synthesis and are reported to play an important role in stabilizing bio-chemical environment inside the cell (Wang and Quinn, 2000, Yamauchi et al., 2001, Vidi et al., 2006). In general longer storage capacity for seeds is an important trait from ecological as well as agricultural perspective. Lipid peroxidation has been reported to be a key factor in seed longevity, as the two factors are inversely proportional to each other (Bailly et al., 1998). The Vitamin E has developed powerful system in plants in response to oxidative stress by significantly enhancing production of antioxidants including catalases, peroxidase, superoxide dismutase and reductase (Farouk, 2011; Semida et al., 2014). These compound molecules tend to detoxify the harmful effects of reactive oxygen species (ROS), generated due to stress, which ultimately disrupts cellular functions and causes membrane degradations. In the present study the comparison between Vitamin E primed and non-primed seeds has clearly shown the difference in concentration of these major compounds and ultimately playing a role in reducing negative impact of aging on seeds and improving seed viability. Our findings is further endorsed by the results of experiments conducted by McDonald (1999) and Sattler (2004).

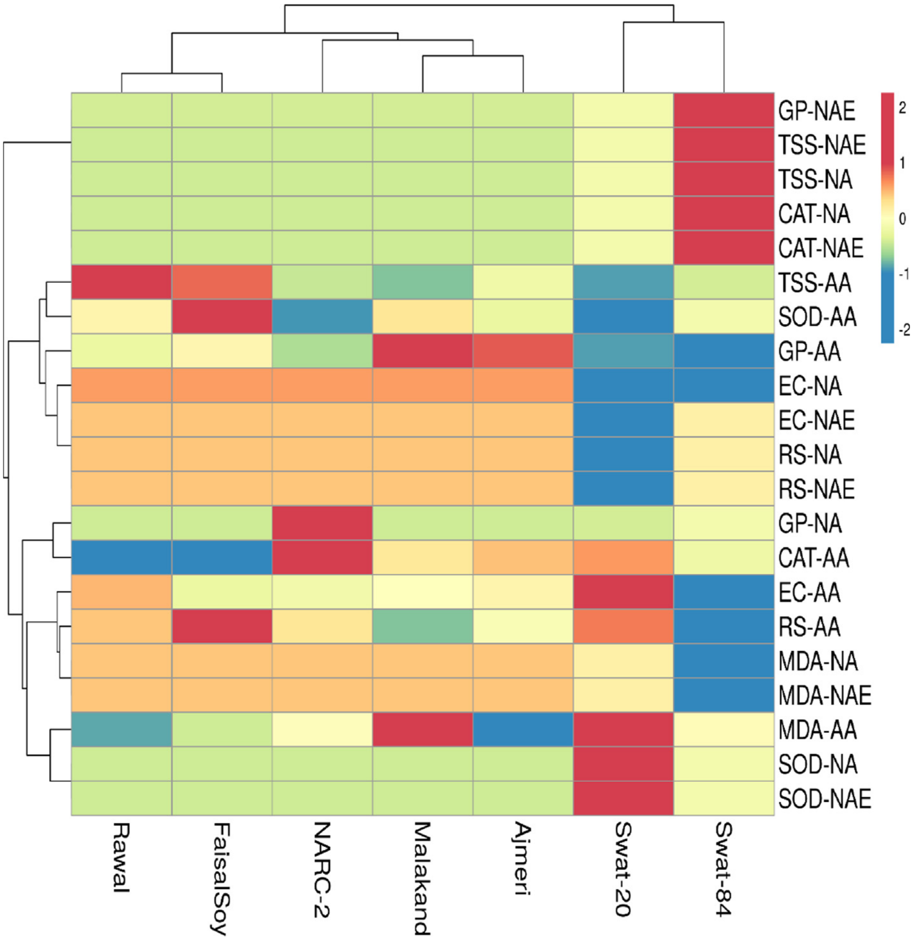

Fig. 4.8. Heat-map for Pearson's Correlation of multiple bio-chemical factors in relation to Natural aging, artificial aging and vitamin E primed seeds under natural aging for each of the given soybean cultivar AA = Artificial Aging, NA = Natural Aging, NAE = Natural Aging Vitamin E treated, GP = Germination Percentage, MDA = Malondialdehyde, CAT = Catalase, TSS = Total Soluble sugars, RS = Reducing Sugars, SOD = Superoxide Dismutase, EC = Electrical Conductivity.

In precise sum up it is concluded that it is evident from our results that aging tends to impart negative influence on seed viability which is evident from statistical analysis of recorded data. Then we have observed the positive impact of Vitamin E treatment on seed health, which highlights its importance as a beneficial seed priming agent for long term storage of seeds.

5. Conclusion

The present study has shown gradual changes in seed under ageing stress conditions with imbalance of anti-oxidant biomolecules leading to ROS accumulation, which initiated process of cellular and enzymatic degradation. As a result, most of cultivar losses seed viability having an ultimate negative impact on final yields. The present study have shown that Swat cultivars (Swat-20 and Swat-84) were resilient to some form of stress induced negative effects, which can further be exploited for better understanding of different cultivar response to ageing stress, and best performing cultivars can be sorted for long term storage, cultivation in stress environment for better yield outputs and food security purposes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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