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Natural Ultra Violet Radialtion on Field Grown Rice (*Oryza sativa* L.) Plants Confer Protection against Oxidative Stress in Seed during Storage under Subtropical Ambience

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Abstract

While heat and humidity are known for causing oxidative macromolecular cell damage related seed deterioration during storage under subtropical conditions, information on effect of UV radiation that is also known to cause bio-molecular as well as direct hit damage in plants is largely lacking. A study involving measurements of UV irradiance in seed storage and also over open fields during seed maturation reveals an inverse correlation between genetically conferred (varietally expressed) content of non-enzymatic antioxidant viz flavonoids (functioning also as successors) and seed deterioration during post harvest storage. The data shows that extent of cell damage and thus seed viability is related to the varietal difference in flavonoids accumulation in mature rice seed. Low storer/viable varieties show lower content of flavonoids and higher oxidative damage in stored seed.

Keywords: ambient UV radiation, antioxidant, flavonoid, seed viability, storage

Abbreviations Used:

β-ME-Beta mercapto ethanol; CAT-Catalase; EDTA-Ethylene diamine tetraacetate; DMSO-Dimethyl Sulphoxide; DNA-Deoxyribonucleic acid; DPPH-1,1-dephenyl-2-picrylhydrazyl; DTNB--5-5'-dithiobis 2-nitrobenzoic acid; LV-Low vigour; h-Hours; HPLC-High performace liquid chromatography; HV-High vigour; O.D-Optical density; N-North; POD-Peroxidase; ROS-Reactive Oxygen Species; SAR-Structure activity relationship; S.D-Standard deviation; SOD-Superoxide dismutase; TCA-Tricarboxylic acid; UV-Ultraviolet

1. Introduction

Orthodox seed, including rice, attain maximum germinability i.e. highest vigour at full maturity. From that time onwards through harvest and post harvest storage, orthodox seeds lose viability/germinability as a function of time and storage environment, Roberts (1973), Leprince et al. (1993). During post harvest storage under ambient environment seeds (viz. the embryonic axes) undergo deterioration in absence of enzymatic repair/ turnover of bio-molecules/antioxidative cell protection, Hendry et al. (1993) leading to loss of seed longevity, Kibiniza et al. (2006). The bio-molecular damage, incurred in the embryonic axis include damage of DNA-Osborne, (1980); Osborne and Boubriak (1994), Boubriak et al. (1997); of membrane-Hallam (1973), Senaratna et al. (1988); of enzymes, Agarwal and Kharluki (1987), Wilson and McDonald (1986a) and also of the tripeptide non-enzymatic antioxidant viz. glutathione, Mitrovic and Bogdanovic (2009) while these earlier studies focused on damage after different periods of seed aging without referring to varietal difference, Sen Mandi and Bhattacharya in 2003 reported that such damages, exhibit varietal difference and are found to be higher in low vigour seed varieties,. Environmental factors causing oxidative stress related loss of seed viability have been reported to be heat and humidity, Elias and Copeland (1994), Walters et al. (2005). It is equally important however to consider another important environmental factor viz. UV radiation that is known to cause oxidative stress as well as direct 'hit' damage of linear macromolecular bonds, Green and Fluhr (1995), Foyer et al. (1994). Delayed germination performance and seedling emergence in mature dry seeds of have been demonstrated to occur under biologically active UV-B radiation, Musil et al. (1998) and also under supplementary UV-C radiation, Torres et al. (1991).

UV radiation is highest in subtropical regions, Turunen and Latola (2005) that encompass rice growing regions of the world. This presents a potential threat for cell molecular deterioration caused loss of viability in embryonic axes and thus viability loss of mature (orthodox) rice seed at low moisture content during post harvest dry storage in this region. Literature in respect of natural UV radiation (albeit low within in-door seed storage) caused seed deterioration during aging is still scanty as has been earlier reflected by Harrington (1960). Scarcity of literature to this effect could be due to the fact that under seed storage cover ambience of UV radiation is expected to be low. A clear understanding of post harvest seed damage vis-à-vis retention of seed viability in seed storage would need to be supported by actual data on measurements of UV radiation over open rice field as well as under seed storage cover.

Whatever the cause of seed deterioration (viz. oxidative stress due to heat, humidity or high energy UV radiation caused hit to linear bio-molecular bonds), cell protectants that may remain undegraded for combating the damaging effect under ambient environment would be relevant for prolonging post harvest seed life.

India, in the subtropical region, lying within 15-35° N latitude is subject to high levels of UV radiation during the months of April-July when the sun shifts to the northern hemisphere. In view of the above a study of the potential for cell protective mechanism in seeds matured and harvested during these months (viz. aush/autumn rice) and stored for 1-2 years in farmers' ambient seed storage would constitute a worthwhile study. A study of seed deterioration vis-à-vis the seed's potential for cell protection during post harvest aging under ambient (including UV) environment is particularly relevant for rice seed since rice husk is composed of 80%-90% compound of amorphous silica, Nayak and Bera, (2009) that is UV transparent, Hsiang et al., (2001) and allows high penetration of UV into rice seed. Tosserams et al., (1997) have demonstrated reduction in germination and seedling performance in *S.jaobaea* seeds under enhanced ambient UV-B radiation during April in dune grassland ecosystem, Netherlands.

For effective cell protection in post harvest dry seed the protective compounds would likely be non linear small molecules that may remain un-deteriorated and functional under UV irradiation and should be effective in mature seed when all other enzymatic repair/protectant activity remain precluded. One such group of cell protective compounds, specifically present in plants viz. the polyphenolic flavonoids appear to be the best contender in this regard. Working on field grown soybean crop Mazza et al. (2000) have provided experimental evidence to establish that in higher plants flavonoids that accumulate in large quantities in the vacuoles of epidermal cells effectively attenuate the UV component of sunlight with minimal effects on the visible region of the spectrum. Biosynthesis of this secondary metabolite viz. flavonoids could occur during (seed maturation associated) dehydration stress (Logeman et al., 2000) as diversion from primary metabolism. It is pertinent to mention here that most of the enzymes in this secondary metabolism pathway are upregulated by UV (Kliebenstein et al., 2002). Thus varietally controlled upregulation of the enzymes would lead to accumulation of varietally different extent of flavonoids in seeds during maturation in open rice field.

Having structure-activity (SAR) relationship that allows:

(i) stabilization under high energy UV radiation via $\pi \rightarrow \pi^*$ transiytion in core nucleus (Cockell & Knowland, 1999) thereby serving as UV screens in epidermal cell.

(ii) phenol-quinone tautomerism in side chain thereby allowing flavonoids to function as (non-enzymatic) antioxidants (Kirsch, 2001), flavonoids, remaining stable through seed storage would be the best contender for cell protection in mature seed for retaining seed viability during post harvest storage.

The present study presents data on:

a) UV irradiance on rice field during seed maturation as well as under ambient seed storage of post harvest rice seed.

b) Flavonoid (non-enzymatic antioxidant) content in different varieties at fresh harvest i.e. cellular content of flavonoids in mature seed.

c) Bio-molecular damage in different rice varieties through two years of aging.

2. Materials and Methods

2.1 Materials

SET I: For unaged fresh seed stocks-Six rice (*Oryza sativa)* varieties differing in seed vigour viz. Patnai-23 (A), Pankaj (B) and Matla(C) (high vigour) and Jaya (D), Jogen (E) and Lalat (F) (low vigour), obtained from Chinsurah Rice Research Station, West Bengal that were grown in Bose Institute Experimental Farm, Madhyamgram for obtaining freshly harvested seeds of all the varieties in the same season. All fresh seeds are homogeneous with respect to germination. Seeds of Aush rice varieties (Autumn) maturation were done during July-August and harvesting at September-October. Three repeat experiments were conducted in the subsequent years i.e. 2005, 2006, 2007.

SET II: For aging experiments seed stocks-Seed stocks stored at 4°C over $CaCl_2$ in a dark cold room served as the unaged 'control' material and those kept in cotton bags under identical ambient environment mentioned in Method section (including spontaneous UV radiation, temperature, moisture content), (for 1 or 2 years) are referred to as aged seeds.

2.2 Experimentation

2.2.1 Conditions at Seed Storage

a) Temperature and humidity of the during storage period: Place: Kolkata (22 34°N, 88 24°E, elevation 20ft msl), West Bengal, India. Kolkata climatologically faces the tropical climate with mean temperature ranging from 13.9ºC to maximum 37ºC, total annual rainfall of ~150 cm with the precipitation ranges from 7.4 to 332 mm during South West monsoon. Relative humidity in monsoon time (June –September) ~80%. The windows of the seed storage room are opened through out the day (6.00 a.m.-5.00 p.m).

b) Determination of UV irradiance at field and at storage condition: The natural ambient UV radiation at rice field under experimentation and at farmer's store room-measured by UV Light meter (Model: UV-340 ISO-9001, CE, IEC1010) for UVA & UVB measurement, Spectrum : 290 nm to 390 nm. UV measurement was also taken in cold room (4ºC) where the control Set I seeds are kept. Sample Time Approx. 1 minute. Three repeat experiments were conducted in the subsequent years i.e. 2005, 2006, 2007 (Table 1).

Table 1. Mean monthly UV irradiance (μWatt/cm^2/min) in open field and in seed store room throughout the year. S. D calculated for 3 and P<0.001

Months	UV irradiance in open field (μWatt/cm^2/min)	UV irradiance in seed store room(μWatt/cm^2/min)
January	1556±0.45	110±0.40
February	1600±0.15	120±0.22
March	1800±0.20	160±0.20
April	2000±0.10	196±0.48
May	2500±0.45	300±0.18
June	2562±0.12	312±0.19
July	2540±0.0.22	300±0.20
August	2500±0.32	286±0.33
September	2255±0.45	212±0.45
October	2000±0.11	196±0.10
November	1775±0.10	175±0.22
December	1600±0.23	146±0.12

2.2.2 Determination of Growth Potential by Measurement of Root/Shoot Length

Three sets of 25 seeds each were arranged in a single row on a moist germination paper, spread on 20 by 20cm glass plate were place at 60º angle in a plexiglass tray with 2 cm of water. All the experiments repeated for six times, maintaining water volume. Root and shoot length were measured daily up to 10 days of incubation on germination plates and histogram was presented root/shoot length (in cm) of seedlings at 72 hrs (Figure 1). S.D was calculated for n=6 and P<0.001.

2.2.3 Determination of Germination /Viability (%V) Percentage

Sets of one hundred seeds from each stock were placed on moist filter paper and maintained at 28±2°C. Germination counts were taken at 24h intervals upto 144hrs. Table 2 shows the germination percentage at 120hrs. S. D was calculated for 6 replicates, P<0.001.

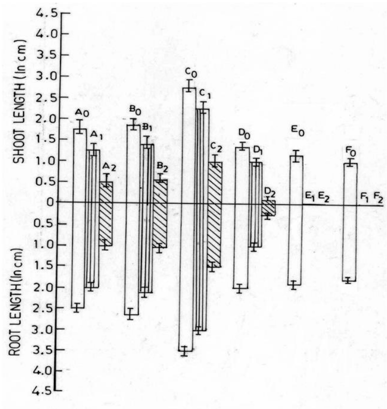

Figure 1. Histogram of root and shoot length of 72 hrs fresh, 1 and 2 years aged rice seedlings grown on germination plates in laboratory at 28±2^{0}C. Unaged seeds designated with 0, 1 year aged seeds designated with 1 and 2 years aged seeds designated with 2.A= Patnai-23, B=Pankaj, C=Matla, D=Jaya, E=Jogen, F=Lalat. S. D was calculated for 6 replicates. P<0.001

Table 2. Table for germination percentage, lipid peroxidation, catalase activity, total UV absorbing compounds, total antioxidant potential,glutathione content and flavonoid content. A=Patnai-23, B=Pankaj, C=Matla, D=Jaya, E= Jogen, F=Lalat. (0 designates unaged, 1 designates 1 year aged and 2 designates as 2 years aged seed stocks). In each cases S. D were calculated for 6 replicates and p<0.001

Rice Varieties	Germination percentage at 120hrs	Lipid peroxidation (Malondialdehyde conc.1/10000 mM/mg fresh wt.)	Catalase activity unit-U=(U/mg of total protein). one unit of CAT converts one µmol of H2O2/min/mg of protein	Total UV absorbing compounds (A_{305nm}/mg fresh wt)	Total antioxidant activity (Percent reduction of DPPH)	Glutathione content (mM/mg total protein)	Flavonoid content by spectro-photometric assay (O.D at 285nnm/mg fresh wt.)
A0	100±0.001	0. 05±0.001	0.669±0.001	0.0695±0.001	80.10%±0.001	0.051±0.001	0.085±0.001
A1	100±0.001	0.1±.0001	0.526±0.001	0.06949±0.001	78.00%±0.001	0.044±0.001	0.085±0.001
A2	60±0.001	0.29±.0001	0.305±0.001	0.06949±0.001	60.00%±0.001	0.032±0.001	0.085±0.001
B0	100±0.001	0.05±.0001	0.676±0.001	0.07124±0.001	87%±0.001	0.054±0.001	0.09±0.001
B1	100±0.001	0.09±.0001	0.53±0.001	0.07124±0.001	85.83%±0.001	0.049±0.001	0.09±0.001
B2	62.1±0.001	0.25±.0001	0.324±0.001	0.07124±0.001	61.2%±0.001	0.038±0.001	0.09±0.001
C0	100±0.001	0.05±0.001	0.68±0.001	0.0754±0.001	94.20%±0.001	0.073±0.001	0.1±0.001
C1	100±0.001	0.07±0.001	0.571±0.001	0.0754±0.001	93.66%±0.001	0.07±0.001	0.1±0.001
C2	69.23±0.001	0.2±0.001	0.424±0.001	0.0754±0.001	65.0%±0.001	0.067±0.001	0.1±0.001
D0	100±0.001	0.05±0.001	0.65±0.001	0.05696±0.001	36.10%±0.001	0.039±0.001	0.06±0.001
D1	95±0.001	0.18±0.001	0.45±0.001	0.056912±0.001	30.00%±0.001	0.023±0.001	0.06±0.001
D2	10±0.001	0.32±0.001	0.186±0.001	0.056912±0.001	20.1%±0.001	0.01±0.001	0.06±0.001
E0	100±0.001	0.05±0.001	0.64±0.001	0.04238±0.001	28.00%±0.001	0.028±0.001	0.032±0.001
E1	63.22±0.001	0.25±0.001	0.321±0.001	0.04237±0.001	20.70%±0.001	0.009±0.001	0.032±0.001
E2	0	0.45±0.001	0.034±0.001	0.04237±0.001	15.4%±0.001	0.002±0.001	0.032±0.001
F0	100±0.001	0.05±0.001	0.633±0.001	0.0332±0.001	20.34%±0.001	0.022±0.001	0.021±0.001
F1	0	0.49±0.001	0.033±0.001	0.0332±0.001	10.25%±0.001	0.0015±0.001	0.021±0.001
F2	0	0.56±0.001	0.033±0.001	0.0332±0.001	8.5%±0.001	0.001±0.001	0.021±0.001

2.2.4 Measurement of Lipid Peroxidation

Lipid peroxidation was assayed according to Health and Packer (1968). 250 mg embryos were homogenized, cenriguged at room temperature in 100mM phosphate buffer pH 7.5 and then treated with the TBA-TCA reagent (0.25% thioberbeturic acid in 10% tricholoroacetic acid), and centrifuged. Absorbance of supernatant was recorded at 532nm indicating malondialdehyde concentration (Table 2). S. D calculated for 6 replicates, P<0.001.

2.2.5 Assessment of DNA Integrity

For viewing the DNA integrity total DNA isolated from dry embryos, Kamalay et al. (1990) was run on 1.0% alkaline agarose gel and observed under UV light, Kamalay et al. (1990). Image analysis scans, Elder and Osborne (1993) of the density of ethidium bromide fluorescence was made of the 1% alkaline agarose gel (Figure 2). S. D calculated for 6 replicates, P<0.001.

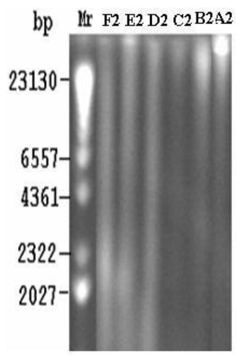

Figure 2. DNA fractionated by 1.0% alkaline agarose gel electrophoresis. A= Patnai-23, B=Pankaj, C=Matla, D=Jaya, E=Jogen, F=Lalat. (0 subscript for freshly harvest seeds 2 subscript for 2 years aged seeds

2.2.6 Spectrophotometric Assay of Total Antioxidant Activity

Total antioxidant activity was assayed (Table 2) according to Rebeiro et al., (2002) by extracting 200mg embryos in Methanol: Water (90:10) and the percent reduction of 0.004% DPPH solution in methanol was assayed at 517nm absorbance by spectrophotometer. S. D calculated for 6 replicates, P<0.001.

2.2.7 Spectrophotometric Assay of Catalase (CAT) (EC 1.11.1.6)

Catalase activity was assayed according to Chance and Maehly, (1955) by extracting 200mg embryo 50mM potassium phosphate buffer (pH 7.0) containing 2mM EDTA, 2mM $MgCl_2$, 3mM NaCl and 10mM β-ME. Assay buffer contains 50mM phosphate buffer (pH 7.0), 15mM H_2O_2 and 75 µg of crude enzyme extract. The decrease in H_2O_2 was followed as decline in absorbance at 240nm. The catalase activity was expressed in µmol of H_2O_2/min/mg of protein (Table 2). S. D calculated for 6 replicates, P<0.001.

2.2.8 Assessment of Total Protein

Total Protein content was measured according to the method of Bradford (1976).

2.2.9 Spectrophotometric Assay for Glutathione

According to the method of Torres et al. (1997) (Table 2), 200 mg embryo was extracted in sodium phosphate buffer pH 7.6 and diluted with 5% SSA (5-Sulfosalicylic acid dehydrate), 400mM sodium carbonate, Phosphate-EDTA dilution buffer and 5-5'-dithiobis (2-nitrobenzoic acid) (DTNB) was added to form the colored product 2-nitro-5-thiobenzoic acid, which was measured at 415 nm. S.D calculated for 6 replicates, P<0.001.

2.2.10 Flavonoid Analysis by High Performance Liquid Chromatography Technique

The flavonoids are extracted and assayed according to the method of Moriguchi et al., (2001). The flavonoids are extracted with 1 ml of a (1:1) (v/v) mixture of dimethyl sulphoxide (DMSO) and Methanol from 100-mg samples. Chromatographic system consisted of Hewlett-Packard 1100 pumps, an automatic sampler, a Hypersil ODS reverse phase column (Hewlett-Packard, 125×ϕ 4 mm r.d.), and UV diode array detector, the flow rate of 1ml/min. The elution buffer contains Phosphoric acid and methanol. The detector was set to measure spectra from 220 to 400 nm & to monitor the eluent at 285 nm. The peak area is recorded by 810-baseline integration report (Figure 3, Table 3). S.D calculated for 6 replicates, P<0.001.

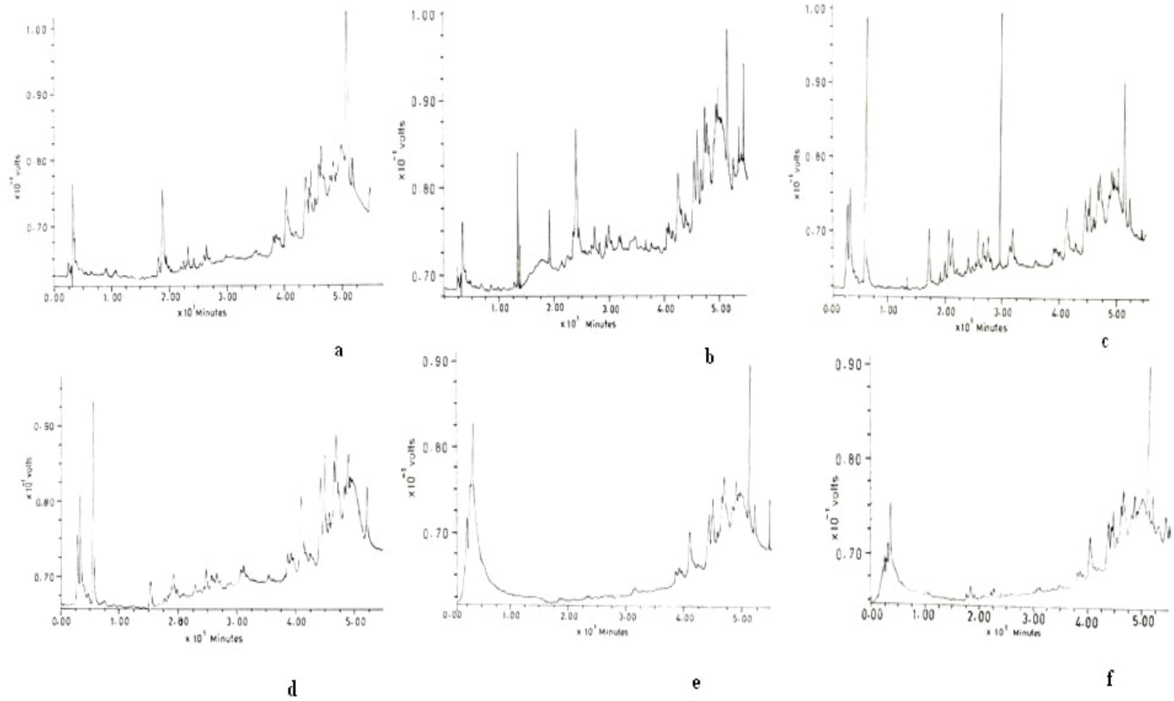

Figure 3. Flavonoid analysis by High Performance Liquid Chromatography (HPLC) analysis. Chromatographic system consisted of Hewlett-Packard 1100 pumps, an automatic sampler, a Hypersil ODS 2 reverse phase column (Hewlett-Packard, 125×ϕ 4 mm r.d.) and UV diode array detector. 3a), b), c), d), e), f) are flavonoid analysis by HPLC technique in six (unaged) rice varieties respectively A=Patnai-23, B=Pankaj, C= Matla, D=Jaya, E=Jogen, F=Lalat

Table 3. Base integration report for flavonoid analysis by HPLC analysis. A=Patnai-23, B=Pankaj, C=Matla, D=Jaya, E= Jogen, F=Lalat. S.D calculated for 6 replicates, P<0.001

Varieties	Total Peak Area (Microvolt-Sec)
A	3880799.2±.02
B	4629317.1±.003
C	5609314.0±.10
D	3603954.1±.001
E	3150264.8±.09
F	2768980.1±.10

2.2.11 Flavonoid Analysis By Spectrophotometric Technique

Total flavonoids content was measured according to Moriguchi et al., (2001)-(Table 2). 200mg of rice embryos were homogenized in 1ml of DMSO: methanol (1:1) (v/v) mixture and centrifuged at 5000 r.p.m for 10mins, supernatant was collected and spectrophotometrically O. D is measured at 285nm. S. D calculated for 6 replicates, P<0.001.

2.2.12 Total UV Absorbing Compounds Analysis:

200mg of each sample were placed in 1.4ml of 99:1 methanol: HCl and allowed to extract for 48hr at -20^0C. Absorbance of extract was read spectrophotometrically at 305 nm for determination of total UV absorbing compounds according to the method of Mazza et al., (2000)--(Table 2). S. D calculated for 6 replicates, P<0.001.

2.2.13 Biostatistical Analysis

In every experiment S. D. was calculated for6 replicates. To test the statistical significance in every cases p-values were tested by using bio-statistical software Meta-analysis 5.3.

3. Result and Discussion

3.1 Natural UV Radiation on Experimental Rice Field during Seed Maturation and Ambient Storage

UV irradiance measurement by UV meter showed a similar trend through consecutive years in 2005, 2006, 2007. The UV radiation was found to be highest during the month of June each year (Table 1) in keeping with the phenomenon of summer solstice when the sun moves to the northern hemisphere and sunshine is maximum within 15-35° N that encompasses India. This data on UV irradiance in Kolkata is similar to that published by the Indian Meteorological Department (www.Indiaenvironmentalportal.org.in). Measurement of Foyo-Moreno et al. (1998) also corroborates these measurements showing highest UV irradiance in the month of June at Granada 37.18 ºN.

In seed storage rooms UV irradiance was found to be similar in trend, albeit lower in value than in the open field (Table 1). During the months of April-July when the sun shifts to the Northern hemisphere, highest UV irradiance is recorded within 15-35°N latitude, (Diffey, 1991, 2002) that includes India, an important rice growing country. During the months of June –July (as per Indian rice cultivation practice) aush (autumn rice) crop is at its seed maturation stage in the open cultivation field.

It is pertinent to mention here that no UV irradiance (0 value) is detected in the closed cold room at 4ºC where fresh seeds were stored for experiments relating to fresh (unaged) seeds.

3.2 Study on Seed Vigour (by Measurement of Root/Shoot Length at 72 Hours) And Seed Viability (Studying Germination Percentage at 120 Hrs)

The present study was conducted on 6 traditional aush (autumn) varieties. In freshly harvested seeds, varieties A, B, C exhibited high vigour features viz. faster seedling emergence and seedling growth rate. The data shows (higher root/shoot length for varieties A, B, C at 72 hours); varieties D, E, F exhibited low seed vigour (Figure 1). Germination rate i.e. seed vigour of all the varieties is seen to decline in aged seeds as a function of time in post harvest storage. With progression of aging the decline is seen to result in loss of viability seen as % reduction in germination count. The data presented (Table 2) demonstrates that the low vigour varieties (D, E, F) lose viability faster than the high vigour varieties (A, B, C). Similar observation has also been reported by Harrington, (1972), Chauhan et al. (1984), Talai and SenMandi (2010). Variability in seed storability potential in different genotypes is suggestive of a genetic control in maintenance of seed viability during post harvest storage, as has also been reported by Berjak and Pammenter (2008).

3.3 Study of Membrane Integrity by Measurement of Lipid Peroxidation

Age related enhancement in lipid peroxidation (that reflects increased membrane permeability) is seen in Table 2. Decrease in unsaturated fatty acid (reflecting membrane deterioration resulting from lipid peroxidation) during ageing has also been reported in 20 years aged clover seed (Flood & Sinclair, 1981). A varietal difference is evident in the data-lower lipid peroxidation in high vigour varieties A,B,C and higher lipid peroxidation in low vigour varieties D, E, F. Effects of such (membrane) deterioration is seen reflected in germination performance (Figure 1, Table 2). Working on six different varieties of orthodox wheat seed (Chauhan et al., 2011) have also demonstrated that membrane damage/lipid peroxidation in aged seed is a varietal phenomenon. It is pertinent to mention here that Kramer et al. (1991) have demonstrated that lipid peroxidation occurs due to UV-B radiation related oxidative stress.

3.4 Status of DNA Integrity

Image analysis scans of the density of ethidium bromide fluoroscence along length of alkaline agarose gel of DNA extracted from 2 year aged stocks shows spread of ethidium bromide fluorescence along the gel length reflecting presence of fragmented single stranded DNA; longer spread of fluorescence indicates higher DNA deterioration in low vigour-viable varieties compared to that in the high vigour-viable varieties (Figure 2). Density of ethidium bromide appearing concentrated at the origin of indicated presence of intact DNA in all the unaged varieties at fresh harvest. Prakash et al. (2007) working with different varieties of soybean (*Glycine max)* have demonstrated that the DNA damage (generated from oxidative stress) was varietal.

3.5 Assay of a Representative Enzymatic Antioxidant Viz. Catalase

Activity of catalase in all varieties at different stages of aging is given in Table 2. At fresh harvest, activity of the enzymes was found to be the same in all varieties; with aging a reduction in enzymatic activity was recorded. Similar observation of age related reduction in antioxidant enzymes has also been reported by Sung and Jeng (1994) in peroxidase (POD) and catalase (CAT) albeit in studies on single variety. Varietal difference in age related reduction in enzymatic antioxidant activity has also been reported by SenMandi and Bhattacharya (2003). In the present study also a varietal trend of reduction in activity was observed (Table 2) - the reduction being greater in low vigour-viable varieties D, E, F than in high vigour-viable varieties viz. A, B, C.

3.6 Assay of Total Antioxidant Activity

Assay of total antioxidant activity including enzymatic and non-enzymatic component that are known to counter damaging effects of reactive oxygen species in living cells is presented in Table 2. The data shows that at fresh harvest, total antioxidant potential is higher in high vigour-viable varieties (compared to that in low vigour-viable varieties).Of particular note is that while enzymatic antioxidant (CAT0 activity remains similar in freshly harvested seed of all high and low vigour varieties, the total antioxidant potential registers a difference between the high vigour and low vigour varieties at fresh harvest. This shows that non enzymatic antioxidant value is higher in high vigour variety than in low vigour variety. This data probably has a bearing on the varietal difference in synthesis of the non-enzymatic antioxidants at fresh harvest when seeds are formed (Table 3). With aging, a reduction in value is evident in all varieties---the reduction being found to be greater in the low vigour-viable varieties. The reduction in total antioxidant potential in aged seed being proportional to the period of aging, suggests that the reduction is due mainly to age related deterioration of enzymatic antioxidant component of the total antioxidant activity in embryonic cells (Table 2). This is corroborated by the data showing reduction in CAT (enzymatic antioxidant) activity in both high and low vigour seeds, through post harvest aging (Table 2).

3.7Assay of Non-enzymatic Antioxidants-glutathione and Flavonoids

Non enzymatic antioxidants that should be contributing to the total antioxidant potential in post harvest seed stored seed would constitute Glutathione, and Flavonoids. Of these glutathione, being a tripeptide, exhibits age related reduction (Table 2), presumably due to deterioration in its linear bonds. The varietal difference observed in the data is a reflection of varietally controlled reduction in antioxidative potential in aged seeds. Ghani (2011) have also demonstrated a varietal difference in reduced glutathione content in aged *Brassica napus* seed. Age related reductions in glutathione have also been reported in naturally aged seeds of *Chenopodium rubrum* L. Mitrovic and Bogdanovic (2009) as well as in artificially (accelerated) aged seeds of *Helianthus annuus* L., Torres et al. (1997). Glutathione, though a non-enzymatic antioxidant, being a tripeptide is subject to spontaneous UV radiation caused integrity loss of the linear bonds. On the contrary, flavonoids, being polyphenolic compounds are not subject to such attacks.

Mature seeds of low vigour varieties show lower values of the non-enzymatic antioxidants (Table 3); the value observed in aged seeds appears mainly due to flavonoids since glutathione is largely destroyed during dry aging of seeds. The low flavonoid content in the low vigour varieties appear as causative factor for the high cell molecular deterioration in low vigour seeds. In the high vigour seeds the protective efficiency seemed much better through periods of post harvest aging. The high vigour seeds showing high value of flavonoids in unaged seed (Table 3) also showed the same value in aged seeds (Table 2). Similarly the low vigour seeds that showed low value in freshly harvested seed retained the same low value even in aged seed without any reduction. No reduction was evident in these compounds through the ageing period; thus aged seeds exhibited essentially same flavonoid content (Table 2). Content of flavonoids under ambient UV radiation measured for continuous 6 years was studied by Robson et al. (2003) in correlation with growth potential of field grown Sphagnum and vascular plants. Lepiniec et al. (2006) reported the role of flavonoid metabolism as responsible for arabidopsis seed viability.

3.8 Study of UV Absorbing Compounds

UV absorbing compounds that includes flavonoids (exhibiting function as sunscreen) is high in amount in mature rice seeds of high vigour-viable varieties; in the low vigour-viable varieties, the value was recorded low (Table 2). Genetic blocks in the synthesis of phenolic sunscreens in phenylpropanoid mutants have been reported to increase susceptibility to UV [in Arabidopsis- Li et al. (1993), Klibeinstein (2002), in Maize-- Stapleton and Walbot (1994)]. Debaujon et al. (2000) have reported that Arabidopsis mutants that lack the ability for flavonoid production exhibit low seed storability/viability trait.

Production of flavonoids has been demonstrated to occur 4 days after pollination during maturation of arabidopsis seed in the field (Debaujon et al., 2000). For autumn-(aush) varieties (here under study) seed maturation occurs during July-August when the field UV irradiance is highest. Enzymes involved in flavonoid biosynthetic pathway that are known to be upregulated by UV radiation-Kliebeinstein et al. (2002) are available in hydrated plant system during growth and seed production in the field. As a corollary, high flavonoid content in freshly harvested seed (particularly of the aush crop) would be due to synthesise during seed maturation. These compounds (unaffected by UV) are found to be retained unchanged in amount through post harvest storage; this is evident in data presented in Table 2. Remaining unchanged in content during aging (Table 2) these sunscreen viz. flavonoids (functioning also as non enzymatic antioxidants) would provide protection from UV caused bio-molecular damage in low moisture containing embryonic axes of stored seed; the higher content of flavonoids in high vigour seeds (and lower content in low vigour seed) suggest a genetic control on flavonoid synthesis during seed maturation. Varieties that have a higher context, viz. high vigour varieties, A, B, C- (Figure 3), would fare better than low vigour varieties D, E, F during seed storage.

4. Conclusion

The subtropical region of the northern hemisphere encompasses several of the rice growing countries of the world. This is also the region that is subject to high UV radiation particularly during the summer months (April-July) that is the rice growing season of autumn (aush) rice with seed maturation in open field occurring during June. During this period sunshine is maximum with 3000h/year and direct sunlight over open field, Cathles et al. (2011). Harvested seed is then stored in seed storage (covered areas) where fluence of UV radiation is lower than that over open rice growing field having an average irradiation of 2562±0.12 μWatt/cm^2/min in June (Table 1) This time of the year this region also has high temperature and humidity that together with high UV radiation present in the environment presents factors that cause high bio-molecular damages. Bio-molecular repair as well as antioxidant and sunscreen activity is thus of utmost importance for perpetuation of orthodox seed life under such stressful circumstances in this region.

As a natural consequence of shutting down primary metabolism in mature orthodox seed during seed maturation when cell moisture content is about to reduce the embryonic cells divert primary metabolism into the secondary metabolite pathway for biosynthesis of flavonoids. During post harvest aging orthodox seed fail to support enzymatic repair/antioxidative activity during post harvest seed aging and the seed is left to cope with adversities of environment till subsequently set out for germination. If, the seed is made to lie in quiescence for too long, deteriorative environment factors viz. heat, humidity, and spontaneous (natural) UV radiation (albeit found to be low under seed storage cover) bring about deterioration the embryonic cells. In absence of repair enzymes/ enzymatic antioxidants such damages accumulate resulting in the final catastrophe i.e., irreversible death of the embryonic cells and thus non viability of the seed. The only redeeming factor under such circumstances is the ability of embryonic cells to avail of cell protection from non enzymatic antioxidants viz. flavonoids that survive the ravages of UV radiation, remaining undegraded and functioning, in 'dry' cells under spontaneous UV radiation. In this context a group of polyphenolic compounds viz. flavonoids known to be present specifically in plants, (particularly in the epidermal layers), serving as sunscreens and also non enzymatic antioxidants could hold cellular damages restrained and thereby allow extension of viability period in post harvest storage.

Varietal difference in total flavonoid content at fresh harvest has been demonstrated in the present study. Interestingly age related reduction in value as noted for all other cell protectants does not occur with flavonoids. No difference occurs in content of flavonoids during post harvest aging (Table 2). This data indicating stability of flavonoids (non-enzymatic antioxidant) through post harvest ambient storage retaining the initially (at fresh harvest) contained high level in high vigour-viable varieties and lower content in low vigour-viable varieties indicates flavonoids as important cellular components for conferring appropriate levels of cell protection during dry cell aging. Remain undegraded during aging in dry orthodox seed, Wu et al. (2002) flavonoids seem to restrain age associated seed deterioration by making adducts with reactive oxygen species and thereby debar the reactive oxygen species from attacking cellular bio-molecules, (Bors et al., 1997). A varietal difference in flavonoid content has also been reported by Yang et al. (2004) in freshly harvested onion seeds. Larson (1997) have demonstrated the role of flavonoids as protective agent during storage against oxidative damage-the extent of protection was varietal.

Using Autumn rice varieties in which seed maturation occurs under high UV radiation in the field we have established the hypothesis that for retention of orthodox seed viability content of cell protection viz. flavonoids is equally important together with macromolecular cell damage accumulation in embryonic axes.

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