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Oxidative stress mitigation studies in two pulse crops

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Abstract

Sulphur dioxide is well studied phytotoxic gaseous pollutant. It is one of the main oxidative gas, which is known for the production of various free oxyradicals during its oxidation from unstable form sulphite (SO_3^{--}) to stable form sulphate (SO_4^{-}) within the life. The oxiradical are initiated by light and mediated by photosynthesis electron transport chain. The generated free radicals react and cause oxidative damage to various biological molecules and cell organelles.

The mechanism of plant tolerance to air pollutants exposure is probably biological rather than biophysical Air pollutant have been shown to affect the level of defense of enzymes as well as than of antioxidant biomolecules present within the plant cells. Protection of sensitive plant species against the oxidative stress may be achieved through various means such as coating the leaf surface and providing physical and/or chemical protection, through alteration of plant metabolism etc.

In the present study, an attempt is made to infuse scavenging potential exogenously in cultivars of two pulse crops namely Lentil (*Lens culinaris* L medic) and Mung bean (*Vigna radiate* L.) using certain antioxidants (α – Tocopherol, ascorbic acid and diphenyl amine). Seeds of both the crops and their selected susceptible cultivars were invigorated exogenously with different antioxidant using dry permeation technique. The plantlets generated were subjected to two different SO_2 concentration (655 and 2620 g/m^3) in open top chambers (OCT) and were evaluated for their response through certain physiological and biochemical parameters. Cultivars JM-721 (Mung bean) and SLC-2 (Lentil) appered to be slightly tolerant than the other respective suseptible cultivars studied (MI–24-91 and Sehore 84 -8). The study trend in general suggests that lower SO_2 concentration was slightly beneficial to both the cultivars of both the crops. All the three antioxidant treatment were comparatively effective in most of the parameters. The treatment affectively however differed for the two cultivars of the same crop. Diphenylamine appeared to be promising in most of the parameters, however most effective was α – Tocopherol followed ascorbic acid.

Keywords : *Oxidative stress*, *Seed invigoration treatment*, *Dry Weight, α–Tocopherol*, *ascorbic acid*, *Sulphur dioxide Protein*

Introduction

Presence of oxygen in the aerobic cellular environment is necessary for aerobic metabolism. This oxygen status poses a constant oxidative threat to cellular structure and different processes. An inevitable result of chloroplast, mitochondrial and plasma membrane linked electron transport is the leaking of electron on to molecular oxygen in plant cells, with the resultant production of reactive, toxic oxygen species or ROS (Rubinstein and Luster, 1993; Asada, 1992; Fridovich, 1995). The imposition of biotic and abiotic stress both can give rise to further increase in ROS levels (Alscher and Hess, 1993; Foyer and Mullineaux, 1994; Dangl *et al.* 1996). Sulphur dioxide is one of most concerned gaseous pollutants since it is potentially a strong free oxyradical generator with in the plant system. According to Halliwell, 1984 there is some kind of enzymatic and/or non-enzymatic antioxidant mechanism, which present within every organism to prevent oxidation of various cellular components. Each and every plant cell has its own capability of self-defense of antioxidant mechanism to cope with the danger posed by presence of ROS by maintaining high redox potential of glutathione reductase (GR) activities. It seems that the ability of a plant to synthesize and maintain reduced glutathione and

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ascorbate may govern its tolerance towards stresses.

Plant cell have embraced the potential of interaction with oxygen for metabolic regulation, (Foyer, 1997). Active oxygen species (AOS) are important metabolites, participating in the metabolism, growth and development of the plant cell. But as the production of this stimulated by environmental stress and since most AOS are highly reactive and lead to perturbation in enzyme activities and membrane damage. They are not compatible with cell function. Also upon further reaction within the cell, can form ROS such as hydroxyl radicals and singlet oxygen, which again frequently are considered to be deleterious and harmful. The study state level of AOS in the cell is determined by the activity of the antioxidant system (Asada 1992, Foyer *et al.*, 1994a) in all situations between AOS the formation consumption is highly controlled. Augmentation of antioxidant defense plays a pivotal role in preventing oxidative stress in plants, (Foyer *et al*., 1994b; Allen, 1995). Plant's survival against toxic oxygen species depends on the variety of small molecules grouped under the general heading of antioxidants. The scavenging/detoxifying potential of each plant species varies from the other. It mostly involves reduction of harmful oxidizing radicals through protection of antioxidant molecules or stimulation enzymes responsible for enhanced production of antioxidants with in the plant system (Rao, 1990; Kumawat, 1990). The inherent scavenging potential can be boosted by exogenous application/infusion of certain antioxidant molecules (Malviya, 1986; Jain, 1993). In the present study an attempt has been made to ameliorate SO_2 toxicity in two pulse crops namely Lentil and Mung bean through exogenous infusion of naturally occurring antioxidant like diphenylamine (DA). A comparison between the actions of naturally occurring and synthetically available antioxidant was also made during the study.

Materials and Method

Plant Material: Two common pulse crops namely Mung bean and Lentil were taken for the present study. Seeds of the different cultivars of both the crop were obtained from Government Agriculture College Indore (M.P.) and Rafi Ahmed Kidwai College of Agriculture, Sehore (M.P.) respectively.Two sensitive cultivars each of the two crops were taken for the study. They were;

Mung bean: cultivers JM-721 and MI 24-91.

Lentil: cultivars SLC -2 and Sehore 84-8.

Experimental setup

Ten healthy seeds of each cultivar of both the crops in their respective growing seasons (Kharif-Mung bean and Rabi-Lentil) were sown in earthen pots separately containing 3kg black cotton soil (clay loam). After 10 days of germination and the plant growth, thinning was carried out and 4 to 5 plantlets were allowed to grow further in each pot. After one month of normal growth the plants were subjected to two concentration of SO_2 (655 and 2620 g/m^3) and a control set was also run simultaneously. Each set was run with three replicates.

SO_2 generation and treatment

SO_2 was generated by bubbling dry air into aqueous solution of Sodium Metabisulphite following Sharma and Thakre (1984). Earthen pots (3 replicates) of each cultivar of both the crops were placed separately in fabricated open top polythene chambers (IX IXI m.) for fumigation and SO_2 was supplied into fumigation chamber through Teflon tubes. The desired concentration of SO_2 in the chamber was maintained and checked at various intervals of the study using toxic gas monitor (TGM-555 CEA, USA).Treatment was carried out at the rate of 6 h/day (7 to 10 AM and 4 to7 PM) for one month. Control set for all cultivars were also run simultaneously. All the past were irrigated timely as required.

Response assay

After one month of SO_2 treatment, different cultivars of both the crops were evaluated for their response against SO_2 toxicity though certain physiological and biochemical parameters following standard procedures as listed below:

1. Stomatal conductance (SC) using steady state porometer Li cor. (1600), USA.
2. Total foliar protein content, following Lowry *et al.*, (1951).
3. Photosynthetic activity using Li cor (6200), USA.

4. Dry weight production study (D.WT.).
5. Seed Invigoration treatment, following dadlani and Agrawal, (1986).

Statistical analysis

The significance of the difference among means was evaluated following Duncan's Multiple Range Test (DMRT). Any value below 95% was rejected.

Results and Discussion

Porometric study stomatal conductance (SC)

All the three antioxidant decrease the SC in case of Mung bean while in lentil an increase was observed, suggesting that an inherent difference exist between the two crops. DA appeared to be better for cultivar JM -721 of Mung bean even though α –tocopherol and DA were better for other cultivars of lentil and Mung bean. Anova suggest that variable SO_2 in Mung bean was highly significant ($p<0.01$), whereas variables, antioxidant and variety were significant ($p < 0.05$). In case of Lentil only variable SO_2 was significant. A similar trend was observed in case of transpiration rate in both the crops. Both the porometric parameters do not suggest any regular trend, only a mixed trend of increase and decrease was observed in different cultivars (Table.1-4).

Table-1. Stomatal conductance (cm/s) of two cultivars of Mung bean treated with different SO_2 concentrations and plant protectants using dry permeation method.

Treatments	Varieties	SO_2 Concentrations (µg/m^3)		
		0.00	655.00	2620.00
Only Acetone	JM 721	1.10±0.56	1.13±0.56	0.92±0.47
	M1-24-91	1.06±0.53	1.16±0.58	0.83±0.42
Tocopherol	JM 721	1.19±0.60	1.34±0.67	1.08±0.54
	MI-24-91	1.11±0.56	1.19±0.60	0.99±0.49
Ascorbic Acid	JM 721	1.12±0.56	1.23±0.61	0.96±0.49
	MI-24-91	1.09±0.54	1.32±0.66	0.95±0.48
Diphenyl amine	JM 721	1.06±0.53	1.24±0.62	0.98±0.50
	MI-24-91	1.02±0.25	1.20±0.60	0.89±0.46

Table-2Analysis of Variance (Split- split-plot Design)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	0.021	0.0107			
SO_2 (A)	2	0.95	0.4751	94.48**	6.94	18.00
Error (A)	4	0.02	0.005			
Sub Plot Analysis						
Antioxidants (B)	3	0.147	0.0491	4.32*	3.16	5.09
A×B	6	0.037	0.0062	0.54	2.66	4.09
Error (B)	18	0.205	0.0114			
Sub Sub Plot Analysis						
Variety (C)	1	0.39	0.392	5.99*	4.26	7.82
A×C	2	0.008	0.0042	0.64	3.40	5.61
B×C	3	0.035	0.0118	1.81	3.01	4.72
A×B×C	6	0.21	0.0035	0.54	2.51	3.67
Error(C)	24	0.157	0.0065			
Total	71	1.642				
CV(a) = 6.5% ; CV(b)= 9.8% ; CV(c)=7.4%						

Table-3- Stomatal conductance (cm/s) of two cultivars of lentil treated with different SO_2 concentrations and plant protectants using dry permeation method.

Treatments	Varieties	SO_2 Concentrations (µg/m^3)		
		0.00	655.00	2620.00
Only Acetone	SLC-2	0.75±0.83	0.74±0.37	0.68±0.34
	Sehore 84-8	0.77±0.39	0.76±0.37	0.71±0.35
α -Tocopherol	SLC-2	0.80±0.41	0.80±0.39	0.70±0.35
	Sehore 84-8	0.77±0.41	0.76±0.38	0.69±0.35
Ascorbic Acid	SLC-2	0.77±0.40	0.77±0.38	0.67±0.33
	Sehore 84-8	0.82±0.42	0.80±0.40	0.74±0.37
Diphenyl amine	SLC-2	0.77±0.39	0.77±0.39	0.67±0.67
	Sehore 84-8	0.82±0.41	0.82±0.41	0.74±0.38

Table-4 Analysis of Variance (Split Split-Plot Design)

Source of Variation	Degree of Freedom	Som of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	0.004	0.0022			
SO_2 (A)	2	0.023	0.0058	8.73*	6.94	18.00
Error (A)	4	0.023	0.0058			
Sub Plot Analysis						
Antioxidants(B)	3	0.011	0.0038	0.82	3.16	5.09
A×B	6	0.002	0.0003	0.07	2.66	4.01
Error (B)	18	0.084	0.0047			
Sub Sub Plot Analysis						
Variety (C)	1	0.013	0.0133	1.78	4.26	7.82
A×C	2	0.002	0.0002	0.11	3.40	5.61
B×C	3	0.019	0.0064	0.86	3.01	4.72
A×B×C	6	0	0	0	2.51	3.67
Error(C)	24	0.18	0.0075			
Total	71	0.44				
CV(a) = 10.0% ; CV(b)= 9.0% ; CV(c)=11.5%						

The Stomatal response to environmental change is important in controlling the observation of pollutants by plant. The reduced stomata aperture resists the entry of pollutant thus preventing their adverse effects on plants (Verma, 2006).

Total foliar protein content

The low SO_2 concentration showed a slightly beneficial effect in Mung bean while the higher SO_2 concentration was deleterious to both the cultivars of each crop (Table. 5-6). The three antioxidant used, appeared to be slightly effective. According to Anova study in Mung bean, variables SO_2 and variety were highly significant and variety were significant ($p > 0.05$).

Photosynthetic activity

Lower SO_2 concentration appeared to be beneficial to both the crops but higher concentration resulted in a significant decrease in the photosynthetic

activity. The decrease was more in susceptible cultivars i.e. MI 24-91 of Mung bean (Table .11) and Sehore 84 -8 of Lentil (Table .12). All the three Antioxidant were significantly effective in reducing the harmful effects of SO_2. Tocopherol and AA were slightly better then DA (Table. 11 - 12). The Anova analysis suggest that in Mung bean the SO_2, variety and antioxidant variables along with antioxidant X variety interactions were highly significant but in Lentil only variable SO_2 was significant.

Dry weight study (D. Wt.)

The overall metabolic state of a plant can be judged by its dry matter accumulation over a period of time, In the present study after SO_2 exposure, the dry matter in terms of dry weight of whole plant showed a slight increase at low SO_2 concentration in both cultivars of each crops, but a reverse trend i.e. decrease in dry weight was observed at higher SO_2 concentration (Table. 15-16). The three antioxidants that were infused in the seeds appeared to be effective and resulted in reducing the decreasing in dry weight that was

Table-5- Total protein content (mg/g. D.wt) of two cultivars of Mungbean treated with different SO_2 concentrations and plant protectants using dry permeation method

Treatments	Varieties	SO_2 Concentrations (µg/m^3)		
		0.00	655.00	2620.00
Only Acetone	JM 721	47.04±0.90	48.26±0.56	36.33±3.31
	M1-24-91	42.91±2.92	41.88±0.43	29.77±12.76
α-Tocopherol	JM 721	47.54±2.03	48.16±0.11	41.01±2.81
	MI-24-91	43.32±1.81	43.50±1.99	34.98±2.43
Ascorbic Acid	JM 721	47.08±4.16	48.36±0.25	40.25±0.18
	MI-24-91	43.74±2.41	44.55±0.13	32.87±0.77
Diphenyl amine	JM 721	47.43±2.03	48.26±0.49	40.72±0.40
	MI-24-91	43.20±2.72	43.76±1.72	32.34±0.02

Table. 6: Analysis of Variance (Split Split-Plot Design)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	16.19	8.0951			
SO_2 (A)	2	1457.284	728.6419	133.79**	6.94	18.00
Error (A)	4	21.784	5.446			
Sub Plot Analysis						
Antioxidants(B)	3	45.857	15.2856	1.32	3.16	5.09
A×B	6	40.518	6.753	0.58	2.66	4.01
Error (B)	18	207.922	11.5512			
Sub Sub Plot Analysis						
Variety (C)	1	505.773	505.7734	48.56**	4.26	7.82
A×C	2	30.956	15.4779	1.49	3.40	5.61
B×C	3	2.977	0.9922	0.1	3.01	4.72
A×B×C	6	7.95	1.3251	0.13	2.51	3.67
Error(C)	24	249.948	10.416			
Total	71	2587.195				
CV(a)=5.5%; CV(b)=8.0%; CV(c)=7.6%						

recorded at higher SO_2 treatment (Table. 15-16). The three antioxidants appeared to be equally effective though there was a slight difference between the three and naturally occurring antioxidant α–Tocopherol and AA had a marginal edge over synthetic protectant DA. The Anova table reveals that SO_2 and variety were highly significant in case of Lentil while only SO_2 variable was highly significant in Mung bean rest other interaction were not very significant.

Table.7: Total (mg/q. D.wt.) of two cultivars of lentil treated with different SO_2 concentration and plant protect ants using dry permeation method

Treatments	Varieties	SO_2 Concentrations (µg/m^3)		
		0.00	655.00	2620.00
Only Acetone	SLC-2	34.87±7.79	32.73±0.20	27.69±0.45
	Sehore 84-8	33-40±7.53	32.17±0.40	24.00±0.91
α -Tocopherol	SLC-2	39.33±4.49	37.52±1.52	34.05±1.05
	Sehore 84-8	35.83±6.92	34.79±1.44	31.60±1.56
Ascorbic Acid	SLC-2	38.36±2.10	36.66±0.60	33.48±1.51
	Sehore 84-8	31.70±4.70	30.97±1.05	26.89±1.37
Diphenyl amine	SLC-2	35.93±2.73	34.09±0.54	29.52±0.55
	Sehore 84-8	37.52±14.22	36.41±0.15	29.80±0.58

Table. 8:Analysis of Variance (Split Split-Plot Design)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	11.68	5.8398	37.51**	6.94	18.00
SO_2 (A)	2	531.398	265.6992			
Error (A)	4	28.336	7.084			
Sub Plot Analysis						
Antioxidants(B)	3	203.27	67.7567	3.23*	3.16	5.09
A×B	6	36.345	6.0574	0.29	2.66	4.01
Error (B)	18	377.464	20.9702			
Sub Sub Plot Analysis						
Variety (C)	1	98.596	98.5964	5.08*	4.26	7.82
A×C	2	4.466	2.2331	0.12	3.40	5.61
B×C	3	147.827	49.2758	2.54	3.01	4.72
A×B×C	6	5.709	0.9515	3.01	2.51	3.67
Error(C)	24	465.982	19.4138			
Total	71	1911.023				
CV(a)= 8.0%; CV(b)=13.8%; CV(c)=13.2%						

Table-9 SH (mole-SH/l. extract) of two cultivars of Mung bean treated with different SO_2 concentrations and plant protectants using dry permeation method.

Treatments	Varieties	SO_2 Concentrations (µg/m^3)		
		0.00	655.00	2620.00
Only Acetone	JM721	0.57±0.16	0.62±0.03	0.93±0.04
	M1-24-91	0.62±0.09	0.68±0.08	1.04±0.07
α -Tocopherol	JM 721	0.58±0.19	0.60±0.05	0.68±0.01
	MI-24-91	0.63±0.22	0.68±0.05	0.87±0.07
Ascorbic Acid	JM 721	0.59±0.11	0.58±0.04	0.70±0.04
	MI-24-91	0.68±0.09	0.74±0.02	0.90±0.04
Diphenyl amine	JM 721	0.62±0.15	0.65±0.03	0.75±0.07
	MI-24-91	0.62±0.09	0.67±0.02	0.84±0.12

Table. 10:Analysis of Variance (Split Split-Plot Design)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	0.004	0.002	56.92**		
SO_2 (A)	2	0.68	0.3402			
Error (A)	4	0.024	0.006			
Sub Plot Analysis						
Antioxidants(B)	3	0.049	0.0161			
A×B	6	0.133	0.0222	2.96		
Error (B)	18	0.099	0.0055	4.05**		
Sub Sub Plot Analysis						
Variety (C)	1	0.156	0.1559	10.85**	4.26	7.82
A×C	2	0.036	0.0179	1.25	3.40	5.61
B×C	3	0.032	0.0107	0.74	3.01	4.72
A×B×C	6	0.004	0.007	0.05	2.51	3.67
Error(C)	24	0.345	0.0144			
Total	71	1.562				
CV(a)=8.0 %; CV(b)=13.8 %; CV(c)=13.2 %						

Table-11 SH (mole-SH/l. extract) of two cultivars of lentil treated with different SO_2 concentrations and plant protectants using dry permeation method.

Treatments	Varieties	SO_2 Concentrations (µg/m^3)		
		0.00	655.00	2620.00
Only Acetone	SLC-2	0.57±0.16	0.65±0.03	0.93±0.03
	Sehore 84-8	0.62±0.09	0.74±0.02	1.99±0.10
α -Tocopherol	SLC-2	0.58±0.19	0.63±0.03	0.81±0.05
	Sehore 84-8	0.63±0.22	0.71±0.05	0.98±0.13
Ascorbic Acid	SLC-2	0.59±0.11	0.64±0.05	0.80±0.04
	Sehore 84-8	0.68±0.09	0.60±0.02	1.04±0.16
Diphenyl amine	SLC-2	0.63±0.15	0.68±0.04	0.93±0.02
	Sehore 84-8	0.62±0.09	0.70±0.03	0.98±0.06

Table. 12:Analysis of Variance (Split Split-Plot Design)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	0.027	0.0133			
SO_2 (A)	2	1.538	0.7691	64.82**	6.94	18.00
Error (A)	4	0.047	0.0119			
Sub Plot Analysis						
Antioxidants(B)	3	0.027	0.000	1.75	3.16	5.09
A×B	6	0.05	0.0084	1.63	2.66	4.01
Error (B)	18	0.092	0.0051			
Sub Sub Plot Analysis						
Variety (C)	1	0.117	0.1168	6.47*	4.26	7.82
A×C	2	0.056	0.0279	1.55	3.40	5.61
B×C	3	0.021	0.0069	0.38	3.01	4.72
A×B×C	6	0.029	0.0048	0.26	2.51	3.67
Error(C)	24	0.433	0.018			
Total	71	2.437				
CV(a)=15.4 %; CV(b)=23.2 %; CV(c)=19.4 %						

α–Tocopherol content

α-tocopherol (Vitamin E) probably is the most important antioxidant that is incorporated into the lipid membrane of the cells (Diplock, 1983). Chloroplast contain large amount of a - tocopherol to protect the membrane against oxidative damage, since most of the fatty acids of chloroplast lipids are unsaturated C_{18} fatty acid (Halliwell, 1984).

It not only, protects against oxygen radicals that might initiate lipid peroxidation of cell membranes, but can also serve as a scavenger of chain propagating free radicals such as lipid peroxyl radical (Niki *et al.*,1984). It does so by donating a hydrogen atom to the lipid peroxy1 or lipoxyl radical, formatting the corresponding peroxide or alcohol respectively thereby breaking the propagating chain reaction (McCay, 1985). α - tocopherol is metabolized to the α - tocopherol radical, which can either be further oxidized to form the α tocopherol quinine (Boguth and Niemann, 1971) or dimmer (Csallany, 1971) or be reduced to generate α - tocopherol (Pascoe and Reed, 1989). Ascorbate and GSH in combination with α - tocopherol can result in synergistic inhibiton of oxidative damage to cell membrances (Niki, 1987), presumably through the regeneration of α - tocopherol. According to Liebler *et al.*, (1986) in bilayer membranes lipid peroxidation was controlled by the ratio of α - tocopherol and ascorbate.In the present study though the initial α - tocopherol content in all the four cultivars of both the crops respectively had marginal differences among themselves (Table17-18). However the dry permeation treatment was carried out only in the susceptible cultivars of the crops, Mung bean (JM-721 and M124-91) and Lentil (SLC-2 and Sehore 84-8). Interestingly, with hardly 2 to 3µg infusion of α-tocopherol through dry permeation could induce reasonably a good amount of tolerance potential in the susceptible test cultivars of both the crops. Though at the end of experiment, in newly set seeds the α - tocopherol content was almost exposed leaves could cause an imbalance between ascorbic acid and dehydro- ascorbic acid resulting in poisoning of specific enzymes and sulphonation of their -SH group.

In the present study the ascorbic acid content initially in seeds of all the cultivars of both the crops was marginally different (Table 17-18). But in the selected susceptible cultivars of both crops Mung been and Lentil, following dry permeation , an increase in ascorbic acid content in the range of 2 to 3 µg was recorded. The trend of foliar increase at different intervals (30 and 45 days) Was

almost same as that was found in α-tocopherol. Also the ascorbic acid content in the newly set seed was almost same as was in the start of the study.Ascorbic acid / ascorbate is perhaps the most important antioxidant in the plants, with fundamental role in the removal of H_2O_2 (foyer,1993). Oxidation of ascorbate occurs in two sequential steps, first producing mono - dehydro -ascorbate and if not rapidly re-reduced to ascorbate, the mono-dehydro-ascorbate disproportionates to ascorbate and dehydro-ascorbate (Asada, 1994) . Ascorbate is not only a potent antioxidant, but is implicated in the pH - mediated modulation of P.S.II activity and its down -regulation associated with zeaxanthin formation (Naubauer and Yamamoto, 1992), which is a potent mechanism for preventing photo - oxidation. In the present study, may be the initial increase in ascorbic acid might have added to the inherent antioxidant at least in the juvenile stages of the corps.

Table.13: Photosynthetic rate (μ mol/m^2/s) of two cultivars of Mung treated with different SO_2 concentrations and plant protectants using dry permeation method.

Treatments	Varieties	SO_2 Concentrations (μg/m^3)		
		0.00	655.00	2620.00
Only Acetone	JM 721	26.31±0.78	26.95±0.78	20.94±0.65
	M1-24-91	23.38±0.22	24.06±0.27	17.25±0.84
α -Tocopherol	JM 721	27.07±1.24	27.94±1.25	22.85±0.60
	MI-24-91	24.73±1.28	25.40±1.58	18.95±1.04
Ascorbic Acid	JM 721	26.70±1.43	27.46±1.15	22.06±1.53
	MI-24-91	22.62±1.00	23.21±1.05	17.46±0.68
Diphenyl amine	JM 721	27.75±1.38	28.50±1.71	23.12±0.66
	MI-24-91	23.45±1.55	24.09±0.89	18.25±0.18

Table. 14: Analysis of Variance (Split Split-Plot Design)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	7.089	3.5443			
SO_2 (A)	2	529.971	264.9857	90.52**	6.94	18.00
Error (A)	4	11.7	2.9274			
Sub Plot Analysis						
Antioxidants(B)	3	40.611	13.537	7.33*	3.16	5.09
A×B	6	8.678	1.4463	0.78	2.66	4.01
Error (B)	18	33.242	1.8468			
Sub Sub Plot Analysis						
Variety (C)	1	218.326	218.3264	186.84**	4.26	7.82
A×C	2	6.757	3.3785	2.89	3.40	5.61
B×C	3	19.316	6.4387	5.51**	3.01	4.72
A×B×C	6	9.017	1.5029	1.29	2.51	3.67
Error(C)	24	26.044	1.1685			
Total	71	912.762				
CV(a)=8.0 %; CV(b)=13.8 %; CV(c)=13.2 %						

Table.15: Photosynthetic rate (μ mol/m^2/s) of two cultivars of Mung treated with different

Treatment	Varieties	SO_2 Concentrations		
		0.00	655.00	2620.00
Only Acetone	SLC-2	13.31 2.73	13.53 0.80	10.04 0.25
	Sehore 84-8	13.41 2.33	13.57 1.13	9.49 0.55
α -Tocophenol	SCL-2	12.71 0.55	12,81 1.36	10.52 1.52
	Sehore 84-8	13.14 2.13	13.27 0.23	10.41 0.13
Ascorbic Acid	SLC-2	13,53 1.05	13.77 0.55	11.12 0.43
	Sehore 84-8	13.53 1.32	13.63 0.53	10.60 0.63
Diphenyl amine	SLC-2	13.41 1.83	13.69 0.17	10.82 0.75
	Sehore 84-8	12.26 1.69	12.36 0.16	5.99 0.61

SO_2 concentrations and plant protectants using dry permeation method

Table. 16: Analysis of Variance (Split Split-Plot Design)

Source of variation	Degree of freedom	Sum of squares	Mean square	Computed F – value	Tabular F -values	
					5 %	1 %
Main plot analysis						
Replication	2	0.054	0.027			
SO_2 (A)	2	142.872	71.4359		66.96	6.94
Error (A)	4	4.268	1.0669			
Sub plot analysis						
Antioxidants (B)	3	4.762	15.875	1.35	3.36	5.09
A× B	6	5.688	0.978	0.83	2.66	4.01
Error (B)	18	21.21	1.1783			
Sub plot analysis						
Variety (C)	1	2.538	2.5375	1.84	4.26	7.82
A×C	2	1.203	0.6017	0.44	3.40	5.61
B×C	3	4.96	1.6533	1.2	3.01	4.72
A×B×C	6	1.018	0.1697	0.12	2.51	3.67
Error (C)	24	33.149	1.3812			
Total	71	221.901				

cv (a) = 15.40 %; cv (b) = 23.20 %; cv (c) = 19.40 %

Table.17: Dry weight (g) of two cultivars of Mung bean traated with different SO_2 concentrations and plant protectants using dry permeation method

Treatment	Varieties	SO_2 Concentrations		
		0.00	655.00	2620.00
Only Acetone	JM 721	2.64 0.40	2.76 0.06	2.16 0.06
	MI- 24-91	2.61 0.57	2.75 0.06	2.05 0.09
α -Tocophenol	JM 721	2.71 0.63	2.81 0.19	2.38 0.15
	MI- 24-91	2.67 0.53	2.80 0.42	2.20 0.21
Ascorbic Acid	JM 721	2.74 0.21	2.87 0.12	2.42 0.36
	MI- 24-91	2.63 0.43	2.76 0.31	2.18 0.19
Diphenyl amine	JM 721	2.75 0.62	2.86 0.25	2.39 0.01
	MI- 24-91	2.65 0.44	2.78 0.06	2.20 0.15

Table. 18:Analysis of Variance (Split Split-Plot Design)

Source of variation	Degree of freedom	Sum of squares	Mean square	Computed F – value	Tabular F – values	
					5 %	1 %
Main plot analysis						
Replication	2	0.145	0.0726			
SO2 (A)	2	3.864	1.9319	18.18	6.94	18.00
Error (A)	4	0.425	1.1063			
Sub plot analysis						
Antioxidants (B)	3	0.134	0.0447	0.56	3.16	5.09
A×B	6	0.07	0.0116	0.15	2.66	4.01
Error (B)	18	1.44	0.08			
Sub sub plot analysis						
Variety (C)	1	0.222	0.224	0.88	4.26	7.82
A×C	2	0.046	0.023	0.09	3.40	5.61
B×C	3	0.41	0.0136	0.05	3.01	4.72
A×B×C	6	0.015	0.0025	0.01	2.51	3.67
Error (C)	24	6.039	0.2516			
Total	71	12.441				

cv (a) = 8.00 %; cv (b) = 13.80 %; cv (c) = 13.2 %

Table.19: Dry weight (g) of two cultivars of Lentil treated with different SO_2 concentrations and plant protectants using dry permeation method

Treatment	Varieties	SO_2 Concentrations		
		0.00	655.00	2620.00
Only Acetone	SLC-2	1.76 0.32	1.80 0.05	1.32 0.08
	Sehore 84-8	1.49 0.19	1.52 0.07	1.02 0.08
α -Tocophenol	SCL-2	1.82 0.11	1.85 0.07	1.50 0.08
	Sehore 84-8	1.41 0.14	1.43 0.07	1.13 0.03
Ascorbic Acid	SLC-2	1.78 0.09	1.81 0.04	1.46 0.04
	Sehore 84-8	1.40 0.19	1.42 0.13	1.12 0.09
Diphenyl amine	SLC-2	1.68 0.48	1.70 0.06	1.38 0.08
	Sehore 84-8	1.51 0.09	1.53 0.08	1.19 0.05

Table. 20: Analysis of Variance (Split Split-Plot Design)

Source of variation	Degree of freedom	Sum of squares	Mean square	Computed F – value	Tabular F – values	
					5 %	1 %
Main plot analysis						
Replication	2	0.019	0.0094			
SO2 (A)	2	1.96	0.9801	25.65	6.94	18.00
Error (A)	4	0.153	0.0382			
Sub plot analysis						
Antioxidants (B)	3	0.022	0.0073	0.28	3.16	5.09
A×B	6	0.087	0.0145	0.56	2.66	4.01
Error (B)	18	0.464	0.0258			
Sub sub plot analysis						
Variety (C)	1	1.623	1.623	42.52	4.26	7.82
A×C	2	0.004	0.0018	0.05	3.40	5.61
B×C	3	0.12	0.0399	1.04	3.01	4.72
A×B×C	6	0.01	0.0017	0.01	2.51	3.67
Error (C)	24	0.916	0.0382	0.04		
Total	71	5.377				

cv (a) = 15.40 %; cv (b) = 23.20 %; cv (c) = 19.40 %

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