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Counteractive impacts of plant growth regulators over uv-b radiation damage on certain physiological and biological aspects in rice crop

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Abstract

The aim of study was to evaluate the appropriate concentrations of plant growth hormones from various concentrations over the UV-B damage on *Oryza sativa* in case of chlorophyll isolation. Seeds of *Oryza sativa* were grown in laboratory for the seed germination and seedling growth and then sown in field plots (A, B, C, D) with appropriate concentrations of plant hormones for the isolation of chlorophyll-a, chlorophyll-b,protochlorophyll. Plot-A of rice crop was treated as control and neither sprayed with growth hormones nor exposed to UVB radiation. Pot-B was treated with UV-B radiation (3-hrs. daily) only. Plot-C was sprayed with IAA concentration of (10^{-7} M), plot-D was sprayed with Kn concentration of (10^{-5} M), along with UV-B radiation in *Oryza sativa*. IAA was found most effective in (10^{-7} M), Kn in (10^{-5}M) in crop of *Oryza sativa* and observed enhancement in the chlorophyll at the germinating seedling stage in the laboratory and field study till to maturity of crop.

Key words: *Oryza sativa* | Plant growth regulators | IAA Kn | Chlorophyll a | Chlorophyll b | Protochlorophyll | UV-B radiation

Introduction

The ozone layer is found at altitudes between 10 and 30 kilometers with a maximum concentration from 19 to 23 km. The total height of the ozone column above any spot on earth is quite small. At standard temperature and pressure, the entire stratospheric ozone layer would have a depth of only 0.3 cm. the depletion of stratospheric ozone caused by increasing human activities have led to an elevation of Ultraviolet-B radiation at high altitudes. Since ambient levels of Ultraviolet-B (UV-B) radiation in the tropics are already high, any further enhancement in UV-B could be of considerable importance in these regions. It may significantly alter plant ecosystems by reducing the productivity of several

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economically important crop plants. India lies in the low ozone belt and is expected to receive high flux of UV-B radiation, which may affect plant growth and metabolism. The increase of ozone depletion raises concerns since the deleterious effects of UV-B radiation have been observed in various plant species (M. Tevini and A.H. Teramura, 1989). Some plants react to UV-B radiation by developing protective mechanisms such as synthesis of UV-B absorbing pigments and thickening of the leaf (Tevini *et al*., 1991). Several recent experiments have shown that UV-B radiation causes increase in the level of cellular reactive oxygen species generating oxidative stress and it is generally accepted'that the mechanism of UV-B toxicity involves oxidative damage (Mazza *et al*., 1999).

Tevini *et al*., (1990 a and b; 1991) observed reduced germination, seedling growth and leaf size in sunflower and maize under high UV-B concentration stimulus. Germination and growth reductions were also found in wild type and stable phytochrome-deficient mutant of cucumber (Ballare *et al*., 1991). The molecular reasons for germination and growth reductions can be attributed to changes in DNA or phytochromes can affect germination and growth by altering their concentration in the growth sensitive tissues and by changing phytochrome dependent processes. Growth in length of root and shoot is related to IAA (indole acetic acid), which absorbs the UV-B range and is readily photo-destroyed by UV-B in vitro and in vivo as shown in sunflower seedlings under low white light conditions. Furthermore, the plastic epidermal cell wall extensibility, which is enhanced in auxin induced elongation, growth was also reduced (Ros, 1990). Peroxidase activity, which can reduce elongation at high activity by different mechanism, was enhanced in UV-B irradiated sunflower (Ros, 1990) and sugarbeet plants (Panagopoulos *et al*., 1990). Another phytohormone, ethylene, which changes elongation of radial growth, is produced to a greater extent in UV-B irradiated sunflower seedlings (Ros, 1990). In UV-B exposed cucumber and bean seedlings, growth could be stimulated by gibberellins (Saile-Mark, 1993; Dhingra *et al*., 2003).

Materials and Methods

The present study was undertaken at the field of R.C. U. Government Post-Graduate. College, Uttarkashi during. The proper study site was located at Purikhet campus of the college near river Bhagirathi. Four plots measuring 1 x 1 in each were fenced by barbed wire to avoid any biotic interference. Certified seeds of cereals crop *Oryza sativa* were procured from extension branch of Indian Agricultural Research Institute, New Delhi.

General Experimental Design: - During laboratory studies following sets were taken into consideration:

Control: Seeds were soaked for 24-hr. in distilled water and placed on moistened filter paper in Peridishes.

UV-B: UV-B radiation was supplied for 3-hr daily by sunlamps (300 W), filtered with quartz interference filters (320 run, ORIEL, USA).

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Growth Regulators: Test solutions of IAA and Kn were prepared in three concentrations viz. 10^{-7}, 10^{-6}, 10^{-5} M (Molarity). Seeds of *Oryza sativa* were soaked for 24-hr in different concentrations of sd k growth regulators. Soaked seeds were placed in paired Petridishes lined with moistened filter paper. One set of Petridish containing soaked seeds was allowed to grow without any UV-B exposure.

Growth Regulators + UV-B: In second set-one from each concentration of different growth regulators was treated with UV-B radiation, for 3-hr daily.

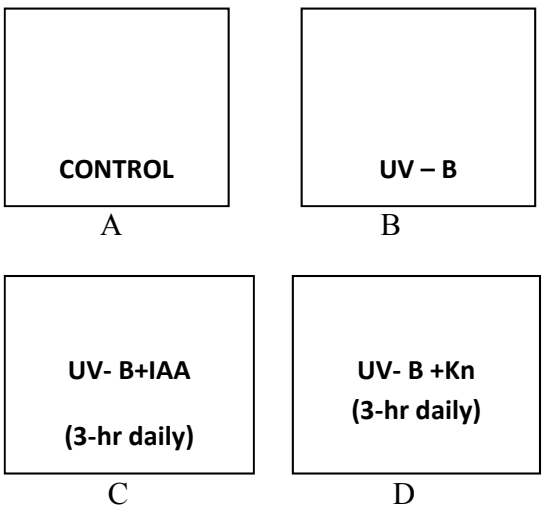
During field study, both the crops were grown in field and the plot was divided by black paper. sheets into four blocks. Each field block was given treatment as:

1. Plot A was taken as control. No treatment was given to the crop of this plot.
2. Plot B was irradiated with 3-hr daily UV-B radiation (24.23 Jm211) by Sunlamps (300 W) filtered with quartz interference filters (320 tim, ORIEL, USA).
3. Plot C was sprayed with IAA ($10^{-.6}$ M concentration) daily alongwith 3-hr supplemental UV-B radiation using the same source.
4. Plot D was sprayed with Kn (10^{-6} M concentration) daily alongwith 3-hr supplemental UV-13 radiation.

General experimental design may be summarized as:

Treatments Concentration	Control	UV-B	IAA			Kn			UV-B+IAA			UV-B+Kn		
		(3-hr)	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}

Treatment of plots in field conditions:



The field for cultivation was prepared before sowing of seeds as proposed by Dhasmana (1984). Pre-soaked seeds of the crops were sown in the experimental plots. The general experimental plan for different treatments was laid after full germination of both the crops (Kumar, 1981; Dhasmana, 1984; Ambrish, 1992; Dhingra, 1999; Neeta Bhatt, 2002).

Chlorophylls:

Fresh leaves (500 mg) were homogenised with 80% acetone, centrifuged at 4000 rpm for 5 minutes. Filtrate was taken out and final volume was made 100 ml, using 80% acetone.

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Optical density was read at different wavelengths viz. 626, 645, 663 mm with the help of Systronics Digital Spectrophotometer. The Chlorophyll COl1tefltS were estimated by the formula given by Koski and Smith (1948) which are expressed below:

Chl a,	mg/l =	12.67 A	663 - 2.65 A	645-0.29	626
Chl b,	mg/l =	23.60 A	645 - 4.23 A	663-0.33	626
Protochl,	mg/l =	29.60 A	626 - 3.39 A	663-6.75	645

Anthocyanins

Anthocyanins during germination of seeds or seedlings were extracted by using the modified method of Mancinelli *et al.* (1975). 500 mg, fresh weight of seedlings was grinded in methanolic HCl (80 ml methanol, 20 ml water, 1 ml HCI). Homogenised tissue was transferred into glass stoppered bottle using appropriate amount of methanolic HCl, stored them overnight in refrigerator. It was centrifuged at 4000 rpm and collected in conical flask. Final volume was made 25 ml with methanolic FTC. Absorbance was taken at 530 nm and 660 nm, with the help of Systronics - Digital UV-spectrophotometer. Calculation was determined by using the formulae given by Mancinelli *et al.* (1975).

As = at 530 — 1/3 A at 660

Where As = Anthocynins

A = Absorbance

Enzymes:

1. **Protease:**

Extraction

Ptotease enzyme was extracted in laboratory conditions from germinating seeds of different treatments of both the crops. One gram of germinated seeds were homogenised in chilled Tris-HCI bufferntrifuged at 5000 rpm for 5 minutes and then supernatant was used as enzyme source. The volume of supernatant was made 25 ml by adding Tris-HCI buffer. All the operations were carried out at 4 to 5°C (Sadasivam and Manickam, 1996).

Assay

Protease activity in extracted material was measured by modified method of Green and Neurath (1954). One ml of extracted material in TrisHC1 buffer, extracted earlier and preserved at 4°C, one ml Of protein solution and one ml of Tris-HC1 was incubated at 40°C for 1 hr. One ml of TCA was added to the above and kept in freezer for 3-hr. After that, the whole solution was centrifuged to get clear supernatant. In supernatant solution, 1 ml of 1.5 N NaOH was added in a separate volumetric flask and final volume was made to 10 ml with distilled water.

One ml aliquot of the above solution was mixed with 5-ml. alkaline copper tartrate solutions and then incubated for 10 minutes at 40° C and then 1 ml. of Folinphenol reagent was added. After 30 minuted, the absorbance of the solution was read at 600 rim. A calibration curve was also prepared following

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above method and utilizing standard amino acids. The released amino acids were measured through comparisons of assayed and standard curves.

Peroxidase:

The extraction of crude enzyme was carried out as followed for protease activity (Sadasivam and Manickam, 1996).

Assay:

One ml aliquot of enzyme extract prepared earlier and preserved at 4°A was mixed with 7 ml distilled water, 2 ml benzidine solution, 2 ml of 6% H202. The optical density of the solution was measured after 1 minute by spectrophotometer using quartz cuvettes at 610 nrn. The activity measured was expressed in A O.D. (difference) (Mahely and Chance, 1967).

Result

Treatments	Chlorophyll a	Chlorophyll b	Protochlorophyll	a/b ratio
A	**0.43**±0.06	**0.37**±0.04	**0.49**±0.02	**11.06**
B	**0.35**±0.03	**0.27**±0.05	**0.38**±0.02	**1.29**
C	**0.36**±0.02	**0.30**±0.02	**0.42**±0.03	**1.20**
D	**0.32**±0.03	**0.36**±0.05	**0.43**±0.04	**0.88**

Table 1: Chlorophyll content at seedling stage after 7 days of germination as affected by UV-B radiation (3-hr daily) individually and combination of IAA and Kn in *Oryza sativa* crop

Treatment	Chlorophyll	15	30	45	60	75	90
	Chlorophyll a	**0.57**±0.04	**0.64**±0.11	**0.77**±0.06	**1.05**±0.12	**0.96**±0.07	**0.93**±0.07
A	Chlorophyll b	**0.62**±0.03	**0.82**±0.08	**0.96**±0.02	**0.12**±0.08	**0.89**±0.08	**0.80**±0.08
	Protochlorophyll	**0.74**±0.62	**0.92**±0.07	**0.92**±0.13	**0.96**±0.13	**0.96**±0.11	**0.72**±0.02
	a/b ratio	**0.91**	**0.81**	**0.80**	**0.87**	**1.07**	**1.16**
	Chlorophyll a	**0.45**±0.05	**0.62**±0.11	**0.67**±0.06	**0.96**±0.11	**0.92**±0.11	**0.78**±0.05
B	Chlorophyll b	**0.56**±0.03	**0.79**±0.06	**0.89**±0.06	**0.95**±0.05	**0.76**±0.07	**0.65**±0.07
	Protochlorophyll	**0.63**±0.04	**0.87**±0.07	**0.82**±0.06	**0.82**±0.04	**0.75**±0.08	**0.62**±0.05
	a/b ratio	**0.80**	**0.86**	**0.75**	**1.01**	**1.07**	**1.20**
	Chlorophyll a	**0.56**±0.06	**0.65**±0.07	**0.75**±0.06	**0.97**±0.11	**0.87**±0.06	**0.89**±0.11
C	Chlorophyll b	**0.60**±0.07	**0.80**±0.11	**0.87**±0.07	**1.02**±0.06	**0.82**±0.06	**0.82**±0.11
	Protochlorophyll	**0.70**±0.04	**0.89**±0.09	**0.89**±0.11	**0.87**±0.14	**0.92**±0.06	**0.76**±0.09
	a/b ratio	**0.93**	**0.81**	**0.86**	**0.95**	**1.06**	**1.08**
	Chlorophyll a	**0.51**±0.05	**0.66**±0.07	**0.69**±0.11	**1.02**±0.06	**0.95**±0.02	**0.87**±0.06
D	Chlorophyll b	**0.62**±0.06	**0.78**±0.11	**0.90**±0.13	**0.96**±0.06	**0.88**±0.03	**0.67**±0.07
	Protochlorophyll	**0.73**±0.07	**0.87**±0.06	**0.91**±0.66	**0.92**±0.06	**0.95**±0.03	**0.73**±0.006
	a/b ratio	**0.82**	**0.84**	**0.76**	**1.06**	**1.07**	**1.29**

Table 2: Chlorophyll contents as affected by UV-Bradiation (5-daily) individually and combition of IAA and Kn in field grown *Oryza sativa.*

Treatments	*Oryza sativa*
A	**0.23±0.06**
B	**0.61±0.07**
C	**0.36±0.09**
D	**0.46±0.07**

Table 3: Anthocynin as affected by UV-B radiation(3-hr daily) indiidually and in combination of IAA and Kn in field grown *Oryza sativa*

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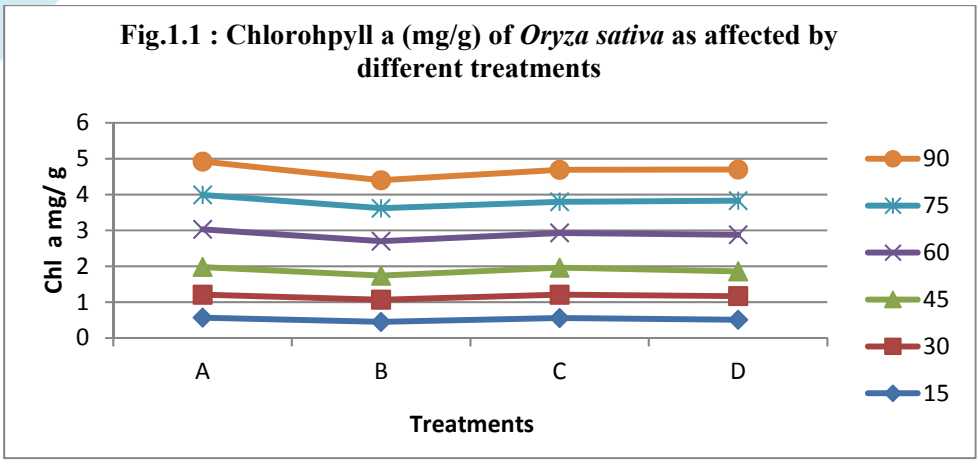

Fig.1.1 : Chlorohpyll a (mg/g) of *Oryza sativa* as affected by different treatments

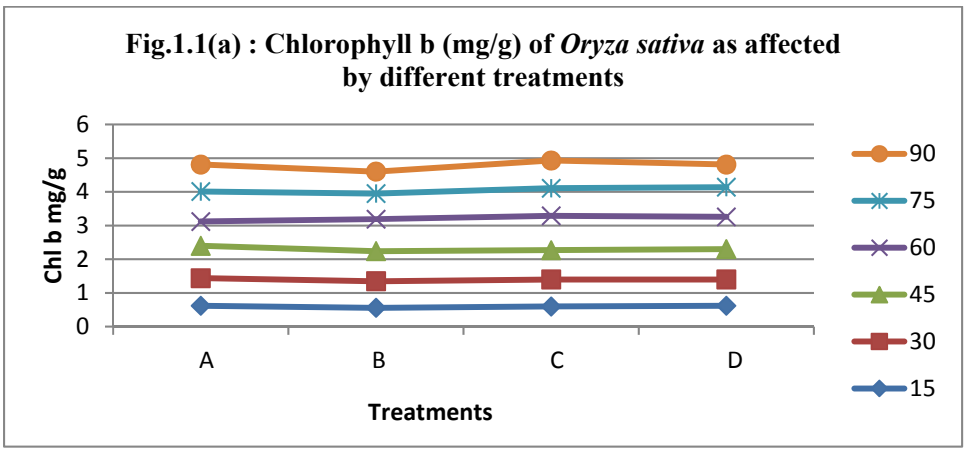

Fig.1.1(a) : Chlorophyll b (mg/g) of *Oryza sativa* as affected by different treatments

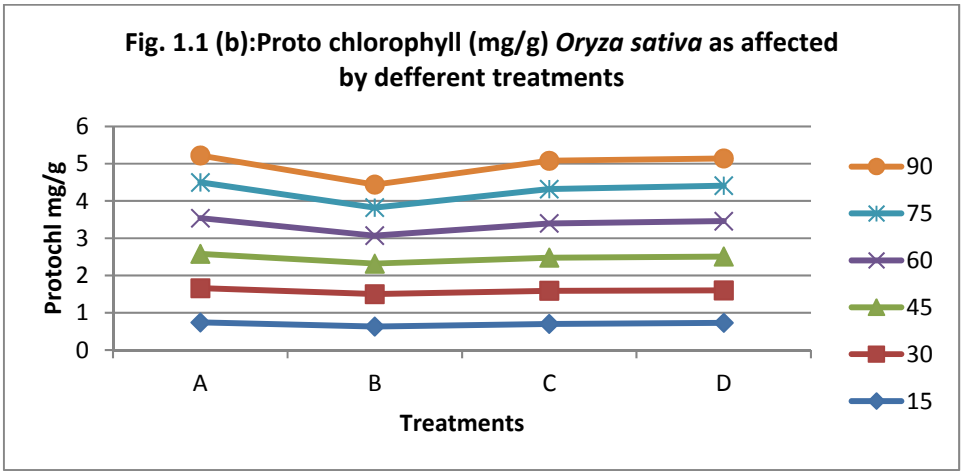

Fig. 1.1 (b):Proto chlorophyll (mg/g) *Oryza sativa* as affected by defferent treatments

Stage	A	B	C	D
Dry	4.200±0.06	-	-	-
6-hr	7.670±0.060	9.067±0.060	10.257±0.70	8.378±0.017
12-hr	8.728±0.070	10.934±0.080	10.537±0.06	10.622±0.02
24-hr	10.288±0.037	16.735±0.43	16.080±0.12	15.689±0.89

Table 4 : Protease activity as affected by UV-B radition (3-hr daily) individually and in combination of IAA and Kn during seed imbibition in *Oryza sativa*

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Stage	A	B	C	D
Dry	4.05±0.07	-	-	-
6-hr	0.425±0.06	0.356±0.05	0.398±0.06	0.387±0.03
12-hr	0.480±0.06	0.687±0.07	0.782±0.09	0.826±0,07
24-hr	0.487±0.07	0.843±0.04	0.927±0.4	0.727±0.072

Table 5 : Peroxidase activity as affected by UV-B radition (3-hr daily) individually and in combination of IAA and Kn during seed imbibition in *Oryza sativa*

Counteracting effects of most of these parameters by different plant growth regulators were also observed during various experiments. These effects are directly or indirectly related to the physiological processes of plants. It can be said that these morphological changes caused by UV-B are the result of physiological distortion. So, in the present study, it is desired to investigate Some physiological parameters in relation to individual UV-B exposure and in combination with certain plant growth regulators.

Chlorophyll Pigment During Seedling Growth

Surface sterilized seeds of Rice was imbibed in water for 6-hr Distilled water washed seeds were transferred to 9 cm petridish (9 diameter cm) for germination and seedling growth studies and treated with UV-B radiation (3-hr daily) alone and alongwith different concentration of plant growth regulators. Chlorophyll a, b and Protochiorophyll were measured after 7 days of growth in both the crops as described in material & methods. The results are presented in table 1.1 for *Oryza sativa*.

A perusal of result in Table 1.1 showed that 3-hr daily UV-B radiation alone and in combination with IAA & Kn affects the different chlorophyll pigments. In control set, the values of these pigments were observed as 0.43±0.06, 0.37±0.04, 0.49±0.02 and 1.16. A marked reduction in chlorophyll pigments except a/b ratio was recorded in plot B, which was subjected to daily 3-hr UV-B exposure. The chlorophyll development was found inhibited by Ca. 19%, 27% and 23% in terms of chlorophyll a, chlorophyll b and protochiorophyll respectively by UV-B treatments. When the seedlings were subjected to PGRs alongwith UV-B treatments, a general promotion was observed in all chlorophyll pigments as compared to UV-B alone. IAA was found to record promotion as Ca. 3%, 11%, 10% in chlorophyll a, chlorophyll b and protochlorophyll and Kn was found Ca. 13%, 13% in terms of chlorophyll b and protochlorophyll. But in case of chlorophyll a, Kn was reported to cause inhibition by ca. 9% as compared to UV-B radiation alone (set B).

Chlorophyll Development During Crop Growth

Effects of UV-B radiation alone and in combination with plant growth regulators on chlorophyll development were also carried out in the s cereals, which were grown earlier for growth pattern studies. Plants for chlorophyll estimation were sampled regularly at 15 days

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interval from seedling emergence up to maturity.

The data set in table 1.2 & fig. 1.2 showed that (3-hr daily) UV-B irradiation alone and in combination of PGRS affected the different chlorophyll pigment in *Oryza sativa*. In control (Plot A), chlorophyll a, chlorophyll b, protochlorophyll and a/b ratio were noticed 0.57±0.04, 0.062±0.03, 0.74±0.04, 0.91 respectively at 15 day stage of growth and found increasing continuously for all the pigments as the crop mature. Plot B indicated a marked reduction in all chlorophyll pigments as compared to control plot. Maximum inhibition in chlorophyll a, chlorophyll b, protochlorophyll and chlorophyll ratio a/b was observed at 15 days, 45 days, 60 days, 75 days stage of growth and inhibited by ca. 21%, Ca. 6%, Ca. 20% and 23% respectively as compared to plot A. The plot C and D reveal promotion of chlorophyll content at all stages of crop growth as compared to individual UV-B treatment (plot B). Kn was observed maximum promotory for different chlorophyll pigment as compared to UV-B treatment alone. The maximum promotion of chlorophyll a, chlorophyll b, protochlorophyll was observed at 75 days stage of growth and recorded as Ca. 15%, Ca. 16%, Ca. 27% respectively as compared to individual UV-B treatment.

Anthocyanins

The effect of UV-B radiation individually and in combination of IAA and Kn on anthocyanin development was studied in rice. Seeds of the crops were presoaked in distilled water in dark for 24 hours and transferred in different petridish for germination and further growth. One petridish carrying seeds of crop was exposed to ordinary white light and treated as control. One petridish of crop was exposedto daily 3-hr UV-B only. Three petridishes of the crops was exposed to UV-B alongwith different plant growth regulators and were carried out in growth chamber. Three days old seedlings were taken for extraction of the anthocyanin as described in material and method.

Ultraviolet—B radiation has positive effect on the accumulation of anthocyanin in *Oryza sativa* seedling. Plant growth regulators viz. IAA (10^{-6}M) and Kn (10^{-6}M) were observed counteracting and lowered down the anthocyanin accumulation induced by UV-B treatment. A perusal three hours daily UV-B exposure caused the marked promotion of anthocyanin pigment in *Oryza sativa.* A promotion of Ca. 165% was recorded in anthocyanin accumulation as compared to control. Different plant growth regulators-viz. IAA and Kn, when given alongwith 3-hr daily UV-B irradiation, were found inhibitory to anthocyanin accumulation level. IAA was recorded much effective to inhibit the accumulation of this pigment and observed Ca. 40% reduction of anthocyanin as compared to UV-B only. Kn showed Ca. 25% reduction as compared to individual UV-B exposure.

Enzymes

Protease:

Effect of UV-B irradiation alone and in combination of plant growth regulators on the protease activity was studied in the seeds of

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Rice. Uniformly selected seeds were soaked in distilled water for 6 hrs, 12 hrs & 24 hrs respectively. Now these presoaked seeds were spread in different petridishes (A, B, C, D). One petridish was kept as control (Neither UV-B nor PGRs), another was exposed to only 3-hr daily UV-B radiation and two petridishes were added with IAA & Kn respectively and exposed to 3- hrs daily UV-B radiation. After providing different treatments, development of protease activity was measured as described in materials & method.

Effect of individual: exposure of UV-B and in combination of PGRs on protease activity in *Oryza sativa* seeds was also studied (table 1.4). After imbibition in water, there was a considerable rise in activity of protease. Data obtained fom petridish A was 7.670±0.060,8.728±0.070,10.288±0.037 at 6 hr, 12 hrs, 24 hrs respectively. Pertridisb B showed an increase by ca. 86% 167% & 87% at 6 hrs, 12 hrs, 24 hrs respectively as compared to control (A). The petridish C & D showed a rise of ca. 104%, 141%, 126& 103%,114%,79% at 6 hrs, 12 hrs, 24 hrs respectively as compared to UV-B treatment alone.

Peroxidase

In order to test the effect of UV-B irradiation individually and in combination of IAA & Kn on peroxides activity, investigations were made on *Oryza sativa* during the course of seed imbibition.

Table 1.5 reveals that peroxidase was linearly increased in case of *Oryza sativa* in individual UV-B treatment. This proxithse activity was reported in control as 0.425±0.06, 0.480±0.06, 0.487±0.07 at 6 hrs, 12 hrs, 24 hrs respectively petridish B studied and recorded an increase of ca. 83%, 143%, 173% at 6. hrs, 12 hrs, 24 hrs respectively as compared to control. Pertidish C & D recorded as increase of Ca. 111%, 113%, 109 and 79% to 32%5 86% at 6 hrs, 12 hrs, 24 hrs respectively as compared to UV-B treatment alone.

Discussion

In The present study, carried out in the laboratory, destruction of chlorophyll, a, b, protochlorophyll and chi a/b ratio was noticed when both the crops were treated with UV-B radiation. The *Oryza sativa* was found more sensitive in chlorophyll reduction. In this crop, chlorophyll a and chlorophyll b were found almost equally reduced due to 3-hr daily treatment of UV-B. When the crops were supplemented with PGRs in addition to the UV-13 radiation, a promotory effect was noted in the present study. Kn (10^{-7}M) was found most promising growth regulator when compared with IAA. Significant reductions in different chlorophyll pigment by UV-B exposure were also investigated by Jain and Goyal (1990), Duysen *et al.* (1985), Sharma *et al.* (1988), Goyal *et al.* (1991), Ambrish (1992) and Dhingra (1999).

The chlorophyll content were also analysed in the field grown crops under the influence of various treatments. In general, it was observed that UV-B inhibits the chlorophyll development throughout the crop age. However, more reduction was recorded in early stages of growth and at maturity. Kn,

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when applied with UV-B radiation, was found to enhance the different chlorophyll pigments level in both the crops, however, the other PGRs also mitigate the adverse effects of UV-B, marginally.

These findings showed the lethal effects of UV-B towards chlorophyll development and repaired by Kn 10^{-6}M. This effect was found variable with the crop skecies. Vu *et al.* (1981, 1983) reported that chlorophyll a/b ratio decreased due to UV-B radiation in soybean but increased in pea.

Tevini *et al.* (1981) concluded that UV-B radiation inhibited the biosynthesis of chlorophyll b than chlorophyll a. Jain and Goyal (1990), while working with lentil crop under field conditions, reported the similar results. They also emphasized that interconversion of protochiorophyll to chlorophyll was retarded. As Kn was found to improve the synthesis of chlorophyll even under increased radiation energy (Purohit, 1988), an improvement in different chlorophyll contents was reported in the present study under similar conditions. One of the measures, which plants develop for the defence towards higher UV-B radiation, is the development of anthocyanin. Present study showed that the crops *Oryza sativa* develop over 165% anthocyanin production as compared to control when treated with 3-hr daily UV-B radiation. A slight decrease in anthocyanin content was noted when the crops were exposed to combined effects of UV-B and different growth regulators. This shows that growth regulators caused insignificant change in the anthocyanin accumulation in plants towards UV-B radiation. Ambler *et al.* (1975) and Bennett (1981) found the accumulation of anthocyanin as a defence of cotton plants against enhanced UV-B radiation. Hashimoto *et al.* (1991) also reported the similar observation, while working with chlorophyll due to enhanced UV-B radiation can be correlated with each other. Enhancement of anthocyanin synthesis can be explained as chloroplast may provide a large reserve pool for the biosynthesis of anthocyanin (Mancinelli *et al.*, 1975). UV-B induced anthocyanins production has also been reported in mustard hypocotyles, corn, wheat and rye coleoptiles (Wellmann, 1982). Arakawa *et al.* (1985) found synergistic increase in anthocyanin production caused by UV-B (312 nm) with white light in apple fruits. Yatsuhashi and Hashimoto (1985) found multi facet action of UV-B photoreepto and phytochrome in the synthesis of anthocyanin using 290 nm (UV-B radiation).

Similar to anthocyanin, flavonoid concentration was also increased, in UV-B treated seedlings after four days of treatment. In contrast, high UV-B fluence increased the flavonoid accumulation (Prem Kumar *et al.,* 2001). According to (Tevini *et al.,* 1990) flavonoid accumulation is regarded as protective mechanism in higher plants to provide against UV-B radiation.

Hence, it is concluded that the UV-B treated seedlings may activate a defense mechanism against UV-B damage by increasing flavonoid. Pal *et al*, (1999) concluded that flavonoid

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concentration can reduce the UV-B penetration and protect the photosynthetic apparatus upto some extent, but it depends upon threshold level which may vary in different species. However, there is also evidence that flavonoids may function in plants to screen harmful radiation, bind phytotoxins and help to regulate the stress response by controlling auxin transport (Shirley, 2002).

This study showed considerable rise in protease and peroxidase activities in the germinating seeds as compared with the pre-existing enzymes in the seeds. Experimental data showed enhancement of protease activity up to Ca. 8% in UV-B exposed germinating seeds as compared to control. When the crop was subjected to combine treatment of PGRs with UV-B, Kn (10^{-6}M) was found most mitigatory which lowered the activity of peroxidase up to 125% while protease activity was lowered slightly when compared to UV-B individual treatment. No significant effects were observed in peroxidase activity with IAA.

Conclusion

1. Experimental studies showed pronounced effect of UV-B exposure and PGRs individually and in, combination on chlorophyll development, of seedlings of the crops. Results of the present study show decrease of Ca. 19%, 27%, 23% for chlorophyll a, chlorophyll b, proto chlorophyll in case *Oryza sativa* respectively after 15 days of seedling growth. When the seedlings were treated with UV-B alongwith IAA it showed as 2%, 11%, 10% for chlorophyll a, chlorophyll b, protochiorophyll. Kn+UV-B showed as 13%, 13%, 9% for chlorophyll a, chlorophylll b, protochiorophyll respectively, when individual treatment of UV-B was given to field grown crops a decline of 21%, 20%, 23% in *Oryza sativa* was recorded for chlorophyl a, chlorophyll b and protochlorophyll. When crops were suppleniented with combination of UV-B and PGRs, an increase in different chlorophyll contents was recorded. Out of the two PGRs, was found to an improved the chlorophyll contents in case of *Oryza sativa*, PGRs were treated with UV-B radiation. Kn was observed maximum promotory for different chlorophyll pigment as compared to UV-B treatment alone. The maximum promotion in chlorophyll a, chlorophyll b and proto chlorophyll was noted as 15%, 16% and 27% respectively.
2. Plants develop anthocynin as a protection pigment against UV-B radiation as evidenced by present as well as other experimental studies. When the seedlings were treated with UV-B, a marked promotion of Ca. 165% of anthocynin pigment was recorded due to UV-B treatment in *Oryza sativa* respectively.
3. The protease activity was also enhanced in germinating seeds of crops due to UV-B radiation. A rise Ca. 86%, 167%, 87% was recorded after 6 hr; 12 hr. 24 hr of soaking in *Oryza sativa* respectively in treated seeds as compared to control.

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4. A marked increase in peroxidase was noted in inhibited seeds of both the crops due to UV-B radiation. An increase of ca. 13%, 96%, 96% *Oryza sativa* after 6 hr, 12 hr and 24 hr of imbibition respectively due to UV-B (3-hr daily) radiation as compared to control.

   All the parameters considered during the present study such as Photosynthetic pigments viz, chlorophyll a, chlorophyll b and protochiorophyll and enzymes Protase as well as Proxidase were also reduced when subjected to UV-B radiation. Effect of UV-B on Wheat and Rice, as far as anthocyanin is concerned was reported an enhancement. It can be assumed after overall studies that accumulation of anthocyanin because of UV-B could act as a screen by absorbing UV-B radiation and in turn protect the chioroplast from UV-B induced damage.

When these most important cereals viz. & Rice were treated with UV-B alongwith PGRS (IAA & Kn), a counteracting effect was reported in all the parameters studied. So, it has been concluded in our study that these plant growth regulators (IAA & Kn) can mitigate the hazardous or deleterious effects caused by UV-B in these cereal crops significantly.

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