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EFFECTS OF UV-B RADIATION ON BIOCHEMICAL COMPOSITION OF *BRASSICA JUNCEA* PR-15 (RAI) AND MITIGATION BY CERTAIN PLANT GROWTH REGULATORS

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ABSTRACT

The seeds of mustard variety of *Brassica junncea PR-15* were imbibed in the water for 6-hrs. and then seeds were washed by distilled water and grown in the petridishes under UV-B exposure, alone and along with different growth regulators such as IAA (10^{-7}), Kn (10^{-5}) and GA_3 (10^{-7}) M (molarities) concentrations were noted during laboratory studies. The seed germination and seedling growth of the *Brassica juuncea PR-15* (Rai) were observed for different concentrations of PGRs, alone and along with UV-B exposure for studies of chlorophyll estimation to & evaluated the appropriate concentrations of these growth regulators, which applied in field studies for the chlorophyll analysis.

Keywords : *Brassica juncea PR-15*, Chlorophyll a, b and Protochlorophyll, GA_3, I AA, Kn & UV-B exposure.

Introduction

The ultraviolet portion of the sunlight is detrimental to plant, animal as well as for human beings and amount of UV-B radiation, reaching at the surface of earth is highly variable as it is influenced by many factors. Ozone is primary UV-B absorbing component of atmosphere with approximately 90% of atmospheric ozone columns being in stratosphere and reminder in the troposphere (WMO, 1995). Clouds, aerosols and surface albedo are also significant factors in determining of UV-B irradiation at particular location and time. UV-B distribution has been significantly affected by anthropogenic activity, through the release of man-made chlorine; bromine compounds such as CFCs, halons and selected solvent, which increased global warming. These undergo sunlight and induced photochemical changes in atmosphere and in stratosphere every CFC molecule atom can destroy up to 100,000 ozone molecules and cause major global concern (Sorg, 1996).

The UV-B exposure has the potential change to damage the key macromolecules and cellular structures, particularly when high doses of UV-B were applied. The studies on UV-B exposure have demonstrated a wide range of photo-biological response among plants with decrease in photosynthesis and plant growth among more sensitive species (Baker *et al.,* 1994). The effects of UV-B on plants conducted mostly under growth chamber and green house condition (Krupa, 1989). The growth of many crop plant species such as soybean, winter wheat, rice, sorghum, cotton, corn and winter mustard etc. species has deleterious effecting by enhanced level of UV-B radiation. The UV-B radiation induced morphogenesis effects in plants and these includes leaf thickening, cotyledons curling, inhibition of hypocotyls, stem & leaf elongation, axillaries branching and shifts in root-shoot ratio (Wilson & Greenberg 1993). The effects of UV-B radiations on plants include reduced biomass allocation & increased flavonoid contents (Kolb *et al.,* 2001).

The effects of UV-B radiation on crop plants, includes reduction in yield and quality, alteration in species competition, photosynthetic activity, susceptibility to disease and changes in plant structure and pigmentation (Tevini & Teramura *et al*., 1989) also observed. These studies show a large number of responses including changes in growth & development, increase in protective pigment biosynthesis, effects on photosynthesis, DNA damage and cellular changes induced by UV-B exposure such as changes in gene expression. These responses are also variable, between species and even within varieties of some species. Changes in photosynthetic pigments were limited to a slight destructive effect of UV-B on the chlorophyll b (Michael Barsing *et al*., 2000).

It has been well known that the plant growth regulators influence the growth and developments of plants. These chemical substances are able to co-ordinate growth among different plant parts or physiological and biochemical processes. The main naturally growth hormone viz. IAA, Kn and GA_3 are able to control many of physiological processes that involved in plant development. Treatments of different growth substances have given remarkably encouraging results in promoting seed germination in tomato, bottle, radish, lettuce, watermelon and a number of other vegetables (Swaminathan *et al.,* 1987). The UV-B radiation can impact on the auxin metabolism. The effects of GA and IAA in dwarf been differ from those reported for dwarf pea.

Cytokinins have also been reported to release dormancy and enhanced germination. The significant increase in content of total chlorophyll with kinetin application as also reported by (Khali & Mandurahi *et al.,* 1989) may also be responsible in photosynthesis. Mok *et al.,* (1994), observed that a large number of plant developmental processes have been found to be influenced by Kn effect on cell expansion, inhibition of leaf senescence, chloroplast, root and shoot branching (Nagel *et al.,* 2001). Therefore, this study was aimed that the mitigatory impacts of plant growth regulators (PGRs) viz. IAA, Kn and GA_3, over the UV-B damage on biochemical composition of *B. juncea PR-15* (Rai) were studied in this investigation.

Materials and Methods

Laboratory and field experiments were conducted in the R.C.U Govt. P.G. College Uttarkashi. Geographically, the District Uttarkashi is located between the central Himalayan region at 30^0 28'to 31^0 28'N latitude and 77^0 49' to 79^0 25'E longitudes at an altitude of 1140 m above mean sea level. The seeds of *B. juncea PR-15* were procured from Seed centre of G.B. Pant University of Agriculture and Technology Pantnagar (Uttarakhand) for the study.

Generel Experimental Design: During laboratory studies, the following sets were taken into consideration:

(A) Control: Seeds of mustard crop *(Brassica junceaPR-15)* were soaked for 24 hrs. in distilled water and placed on moistened filter paper in Petridishes.

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

(C) Growth Regulators: Test solution of IAA, Kn and GA_3 were prepared in three concentrations viz.10^{-7}, 10^{-5} & 10^{-7}M (molarities) in *B. juncePR-15.* The seeds of *B. juncea PR-15* were soaked for 24 hrs. in different concentrations of 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.

(D) Growth Regulators + UV-B: In second set, one from each concentration of different growth regulators was sprayed with UV-B radiation, for 3-hrs. daily.

Treatments		**UV-B**	**IAA**			**Kn**		**GA_3**			**IAA+UV-B**				**Kn + UV-B**		**GA_3+UV-B**			
Concentration	**Control**	(3-hrs)	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}

Field study: During field study, mustard crop of *B. juncea PR-15* were grown in field and the plots were divided by black paper sheets into five blocks. Each field block was given treatments as follows:

Treatments of field plots

1. 1. In plot-A, mustard plant (*B. juncea PR-15*) species was taken as control. No treatments were given to crop of this plot.
2. Plot-B was exposed to 3-hrs. daily UV-B radiation (24.23 Jm^{-2} Z^{-1}) by Sunlamps (300W) filtered with quartz interference filters (320 nm, ORIEL, USA).
3. Plot-C was sprayed with IAA (10^{-7} M) concentration daily, along with 3-hrs supplemental UV-B radiation using the same source.
4. Plot-D was sprayed with Kn (10^{-5} M) concentration daily, along with 3-hrs. Supplemental UV-B radiation by using the same source.
5. Plot-E was sprayed along with GA_3 (10^{-7} M) along with 3-hrs. Supplemental UV-B radiation, using the same source as above.

Results

Chlorophyll analysis during seedling growth in laboratory, the surface sterilized seeds of mustard crop such as Rai (*B juncea PR-15)* were imbibed in the water for 6-hrs. and then seeds were washed by distilled water and transferred to Petridishes, for the seed germination and seedling growth studies and exposed with UV-B radiation (3-hrs. daily), alone and along with various concentration of plant growth regulators. Chlorophyll a, b and protochlorophyll were measured after 7 days of growth in different treatments.

An observation of data in table 1.1, & Fig. 1.1 showed that in *B. juncea PR-15*, the chlorophyll a (mg/pl), chlorophyll b (mg/pl), protochlorophyll and ratio of chlorophyll a/b was studied in control set-(A) and amounted as 0.44±0.04, 0.43±0.03 0.95±0.05 & 1.02 respectively. When the seedlings were studied with UV-B radiation alone, it showed a marked decline in contents of chlorophyll pigments except a/b ratio. Chlorophyll development was observed and inhibited by ca. 16%, 24%, 6% & 33% respectively in terms of chlorophyll a, b protochlorophyll & chlorophyll a/b ratio under UV-B treatments. When the seedlings were exposed to UV-B exposure along with different PGRs, a general promotion was recorded in all chlorophyll pigments as compared to individual treatment of UV-B exposure. The IAA was found to record promotion as ca. 2.6%, 10%, 4.3% & 7.7% respectively in chlorophyll a, b, and protochlorophyll and chlorophyll a/b ratio. Kn was found to recorded promotion as ca. 16.6% for chlorophyll b, 2.2% for protochlorophyll and 46% for a/b ratio respectively & GA_3 showed a promotion as ca. 2.7% for chlorophyll a, 13.4% for chlorophyll b, 6.4% for protochlorophyll & 46% for a/b ratio respectively. But in case of chlorophyll a, the Kn was identified to cause inhibition by ca.11% as compared to UV-B radiation alone.

Table 1.1: Chlorophyll content at seedling stage after 7 days of germination as affected by UV-B radiation (3 hrs. daily), individually and in combination of IAA, Kn and GA_3 in *Brassica juncea PR-15*.

Treatments	Chlorophyll a	Chlorophyll b	Proto-chlorophyll	a/b ratio
A	**0.44±**0.04	**0.43**±0.03	**0.45**±0.05	1.02
B	**0.34±**0.01	**0.36**±0.02	**0.40**±0.03	0.94
C	**0.39**±0.03	**0.40**±0.04	**0.44**±0.04	0.97
D	**0.38**±0.06	**0.35**±0.05	**0.40**±0.01	1.08
E	**0.35**±0.02	**0.32**±0.01	**0.41**±0.05	1.09

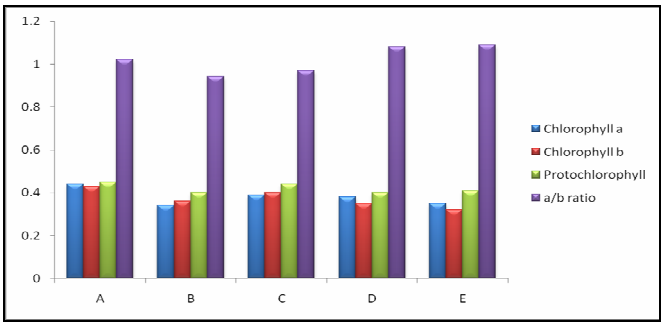


Fig 1.1: Chlorophyll content at seedling stage after 7 days of germination as affected by UV-B radiation (3 hrs. daily), individually and in combination of IAA, Kn and GA_3 in *Brassica juncea PR-15*.

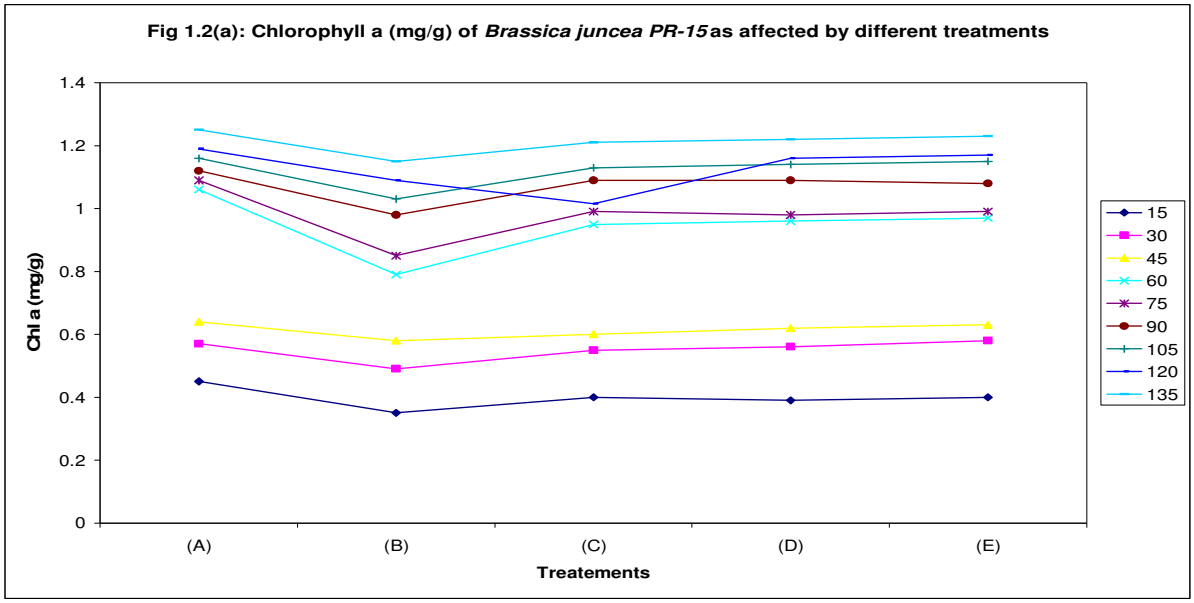
Effects of UV-B exposure alone and along with some plant growth regulators on chlorophyll development during crop growth were also observed in same variety of mustard crop, which were grown for growth patterns studies. For the chlorophyll estimation, plants were selected regularly at 15 days interval from the seedling emergence upto maturity. Data presented in table 1.2 and Fig. 1.2 (a), (b) & (c) showed that, 3 hrs. daily UV-B irradiation was supplied alone and along with different plant growth regulators (PGRs) affected the different chlorophyll pigments in *B. juncea PR-15*. The chlorophyll a, chlorophyll b, protocholorophyll and chlorophyll a/b ratio were noticed as 0.45±0.04, 0.52±0.02, 0.67±0.07 & 0.86 respectively in control (plot-A), at the 15 day interval of crop growth and increased continuously upto maturity and amounted as 1.25±0.19, 1.20±0.12, 1.21±0.08 mg/pl & 1.04 for all chlorophyll pigments as crop matured. The plot-B, showed a marked reduction in all chlorophyll pigments as compared to control plot. Maximum inhibition in chlorophyll a, b, protochlorophyll & chlorophyll a/b ratio was reported at the 30th day & decreased by ca. 15%, 8%, 7%, 8%; at 60th day ca. 26%, 29%, 23%, 6% and at 90th day ca. 13%, 17%, 19%, 3% respectively as compared to control. When plot C, D and E were exposed to UV-B, along with PGRs, a general promotion was observed in all chlorophyll content up to maturity as compared to UV-B treatment.

The IAA concentration of hormone showed that the maximum promotory effects at 15th day stage of growth & continuously increased up to maturity & promoted by ca. 14%, 8%, 9% & 9% respectively in terms of chlorophyll a, b, protochlorophyll & chlorophyll a/b ratio as compared to UV-B exposure only. When Kn and GA_3 concentrations were applied, maximum promotion was observed in terms of chlorophyll a, chlorophyll b, protochlorophyll and chlorophyll a/b ratio at 120th day & maturity stage increased by ca. 7%, 6.7%, 11% 9% & 7%, 6%, 9%, 9% respectively as compared to UV-B treatment only.

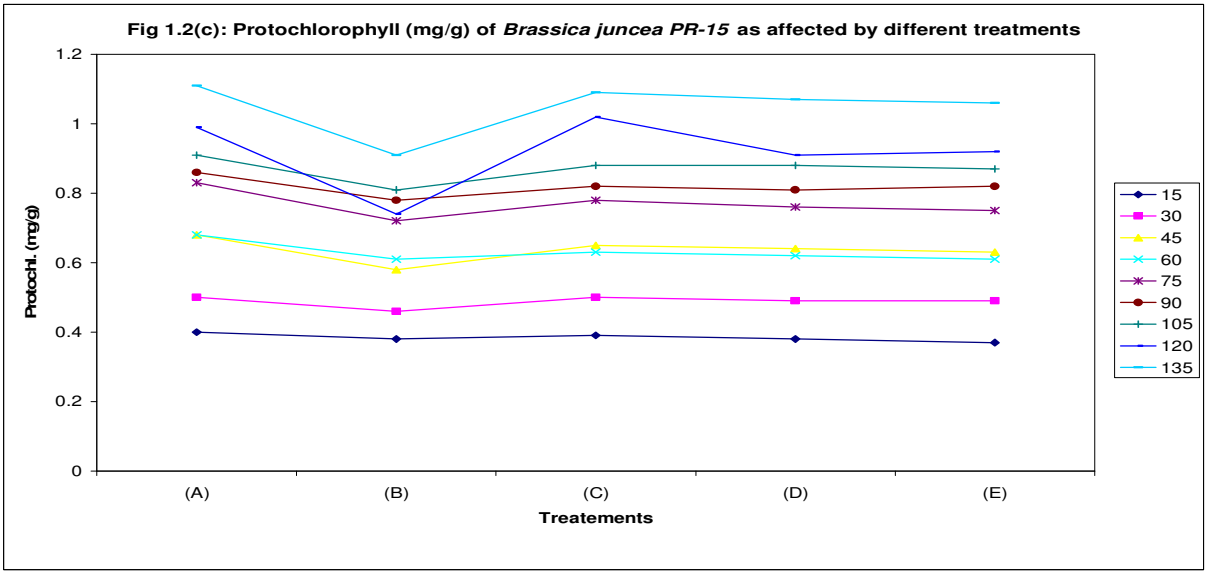
Table 1.2: Chlorophyll contents as affected by UV-B radiation (3 hrs. daily), individually and in combination of IAA, Kn and GA_3 in field grown crop of *Brassica juncea PR-15*.

Treatments	Chlorophyll	15	30	45	60	75	90	105	120	135
A	Chlorophyll a	**0.45** ±0.04	**0.57** ±0.12	**0.64** ±0.01	**1.06** ±0.12	**1.09** ±0.14	**1.12** ±0.11	**1.16** ±0.15	**1.19** ±0.17	**1.25** ±0.19
	Chlorophyll b	**0.52** ±0.02	**0.61** ±0.01	**0.79** ±0.02	**1.05** ±0.11	**1.07** ±0.12	**1.10** ±0.09	**1.14** ±0.11	**1.17** ±0.18	**1.20** ±0.12
	Protochlorophyll	**0.67** ±0.07	**0.73** ±0.03	**0.89** ±0.09	**1.09** ±0.15	**1.08** ±0.11	**1.11** ±0.08	**1.15** ±0.12	**1.16** ±0.09	**1.21** ±0.08
	a/b ratio	0.86	0.93	0.81	1.01	1.02	1.08	1.09	1.1	1.04
B	Chlorophyll a	**0.35** ±0.05	**0.49** ±0.04	**0.58** ±0.08	**0.79** ±0.09	**0.85** ±0.04	**0.98** ±0.01	**1.03** ±0.09	**1.09** ±0.11	**1.15** ±0.13
	Chlorophyll b	**0.45** ±0.03	**0.57** ±0.07	**0.65** ±0.05	**0.75** ±0.07	**0.84** ±0.02	**0.92** ±0.03	**1.01** ±0.03	**1.08** ±0.09	**1.11** ±0.70
	Protochlorophyll	**0.59** ±0.09	**0.68** ±0.08	**0.78** ±0.08	**0.85** ±0.05	**0.90** ±0.09	**0.91** ±0.04	**1.05** ±0.04	**1.06** ±0.05	**1.10** ±0.50
	a/b ratio	0.77	0.86	0.89	1.05	1.06	1.06	1.09	1.1	1.03
C	Chlorophyll a	**0.40** ±0.01	**0.55** ±0.13	**0.60** ±0.06	**0.95** ±0.03	**0.99** ±0.09	**1.09** ±0.15	**1.13** ±0.12	**1.015** ±0.16	**1.21** ±0.15
	Chlorophyll b	**0.49** ±0.05	**0.59** ±0.01	**0.69** ±0.07	**0.89** ±0.09	**0.94** ±0.04	**1.08** ±0.13	**1.11** ±0.09	**1.16** ±0.12	**1.16** ±0.11
	Protochlorophyll	**0.63** ±0.03	**0.69** ±0.09	**0.85** ±0.05	**0.92** ±0.02	**0.95** ±0.05	**1.09** ±0.12	**1.12** ±0.08	**1.17** ±0.13	**1.17** ±0.07
	a/b ratio	0.81	0.93	0.83	1.06	1.05	1.1	1.12	0.99	1.04
	Chlorophyll a	0.39 ±0.02	0.56 ±0.14	0.62 ±0.05	0.96 ±0.04	0.98 ±0.08	1.09 ±0.14	1.14 ±0.13	1.16 ±0.17	1.22 ±0.16

D	Chlorophyll b	0.48 ±0.04	0.59 ±0.02	0.74 ±0.06	0.89 ±0.08	0.93 ±0.04	1.08 ±0.12	1.12 ±0.09	1.15 ±0.13	1.17 ±0.12
	Protochlorophyll	0.62 ±0.02	0.68 ±0.09	0.86 ±0.06	0.93 ±0.03	0.96 ±0.05	1.10 ±0.11	1.13 ±0.07	1.18 ±0.14	1.18 ±0.09
	a/b ratio	0.81	0.94	0.83	1.07	1.05	1.09	1.11	1.08	1.1
	Chlorophyll a	0.40 ±0.04	0.58 ±0.15	0.63 ±0.06	0.97 ±0.07	0.99 ±0.09	1.08 ±0.15	1.15 ±0.01	1.17 ±0.18	1.23 ±0.17
E	Chlorophyll b	0.49 ±0.09	0.60 ±0.03	0.75 ±0.05	0.89 ±0.09	0.94 ±0.04	1.09 ±0.13	1.13 ±0.09	1.16 ±0.14	1.18 ±0.13
	Protochlorophyll	0.63 ±0.03	0.67 ±0.08	0.87 ±0.07	0.94 ±0.04	0.95 ±0.03	1.11 ±0.11	1.14 ±0.04	1.19 ±0.13	1.19 ±0.07
	a/b ratio	0.81	0.96	0.84	1.08	1.06	0.99	1.01	1.08	1.04



Chlorophyll a (mg/g) of *Brassica juncea* affected by different treatments



Protochlorophyll (mg/g) of *Brassica juncea* as affected by different treatments

Discussions

Present study was carried out in laboratory & field to observed the destruction of chlorophyll, a, b, protochlorophyll & chlorophyll a/b ratio were noticed, when crop was treated with UV-B radiation. Chlorophyll a & chlorophyll b were found almost equally reduced due to 3 hrs. daily UV-B treatment. When the crop was supplemented with PGRs in addition to UV-B radiation, a promotory effect was noted in present study. The IAA and GA_3 were found most promising growth regulators, when compared with Kn. Significant reductions in different chlorophyll pigment by UV-B exposure were also investigated by Jain & Goyal (1990) & Ambrish *et al.,* (1992). The chlorophyll content was also analyzed in 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, when applied with UV-B radiation, it was found to enhance the different chlorophyll pigment level in mustard variety of *B. juncea PR-15* however, other PGRs also mitigate the adverse effects of UV-B, marginally.

These findings showed that, lethal effects of UV-B towards chlorophyll development and repaired by Kn (10^{-5} M). This effect was found variable with the crop species. Vu *et al.* (1981) 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 *et al.* (1990), while working with lentil crop under field conditions, reported similar results. They also emphasized that interconversion of protochlorophyll to chlorophyll was retarded. Kn was found to improve the synthesis of chlorophyll even under increased radiation energy observed by Purohit *et al.,* (1988), an improvement in different chlorophyll contents was reported in present study under similar results.

Conclusion

As noted the individual treatment of UV-B radiation in *B. juncea PR-15,* inhibited the chlorophyll pigments viz. chlorophyll a chlorophyll b protochlorophyll and chlorophyll a/b ratio. When treatment with IAA, Kn and GA_3 were showed a promotion or enhancement in all chlorophyll pigments. Therefore, plant growth hormones (PGRs), would show the maximum mitigation against UV-B induced deleterious effects on chlorophyll a, chlorophyll b protochlorophyll & chlorophyll a/b ratio.

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