# Influence of Ultraviolet-C Radiation on Biochemical Compositions and Genetics of *Capsicum* Plants

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**ABSTRACT** Chilli or Pepper (*Capsicum* species) is an essential and critical plant shown as vegetable cum spice crop grown for the ornamental, medicinal and pharmaceutical applications. The present paper aimed to understand the relative changes in biochemical composition of the plants because of impacts of the induced mutation and amplified fragment length polymorphism (AFLP) investigation can be utilized to assess the alteration in the DNA structure. Minimum amount of proteins (799.997 µg/ml), carbohydrates (39.13 µg/ml) and free fatty acids (673.2 µg/ml) were found in very high intervening mutated plants. Maximum percentage of inhibition by the peroxidase activity (0.1685 µg/ml), catalase activity (0.078 µg/ml), diphenyl-1-picrylhydrazyl (79.44±1.61 µg/ml) activity and 2, 2'- azino-bis (3-ethylbenzoithioazoline-6-sulphonic acid) (97.02±0.45 µg/ml) activities were found in profoundly exposed mutated plants compared to the normal *Capsicum* plant species. A dendrogram representing the similarity in DNA bands of *Capsicum* plants in different time intervals of UV-C radiations showed significant negative affinities. This study encourages us in understanding an induced mutation by UV-radiation on the Chilli plant leaves and its impact on plant cell creation and DNA structure. Therefore, this study brings mutation prompts diverse sorts of basic variations from the norm in cell organelles and its structure of the plant sources.

**KEYWORDS:** ABTS assay, Catalase activity, DPPH assay, Peroxidase activity, Ultraviolet radiations.
**Received** 18 April 2021 **Revised** 11 June 2021 **Accepted** 20 June 2021 **Online** 30 June 2021

**Original Article**



## INTRODUCTION

In the tropical, subtropical and temperate regions of the world, Pepper or Chilli (*Capsicum* species) is an essential and critical plant shown as vegetable cum spice crop that grown for the ornamental, medicinal and pharmaceutical applications (Hazra *et al.,* 2011). The genus *Capsicum* lies within the Solanoideae sub-family of the Solanaceae family (tribe Solaneae and subtribe Capsicinae) belonging to the order Solanales and believed to have its ancestral origins in Bolivia of the tropical South American region. Amongst the most critical vegetable and spice crops esteemed for its smell, taste, flavour and sharpness and, enormous source of nutrients of vitamins A, C and E constituted in the *Capsicum* species L. (2n = 24). This species is the most widely recognized and broadly developed of the five domesticated *Capsicum*s. These species incorporate *C. annuum, C. frutescens, C. baccatum, C. pubescens* and *C. chinense*. The genus *Capsicum* belonging to the family Solanaceae incorporates 27 species comprising of five domesticated species in particular *C. annuum* L., *C. baccatum* L., *C. chinensis* Jacq., *C. frutescens* L. and *C. pubescens* Ruiz and Pavan (Onus and Pickersgill, 2004; Ince *et al.,* 2009).

Mutation has been depicted as a sudden change in the hereditary material of the living cells. Since mutation deals with hereditary material, mutations can be found in every single cell constituent that convey DNA, i.e., the nucleus, the mitochondria and (for plants) plastids. It is the sudden changes in the morphological, cytogenetical and physiological traits of a living beings (including plants and animals) because of the ecological conditions (natural or induced) for example, light, carbon dioxide, oxygen and water, UV radiation, and so forth.

The physical means that origin harm to the DNA molecules of a living being are stated to as physical mutagens or mutagenic radiations. Mutation also is caused by the methods for the ionizing and non-ionizing (UV and gamma rays) radiations. Moreover, UV radiation is the portion of the non-ionizing region of the electromagnetic spectrum that contains roughly 8-9% of total solar radiation (Coohill, 1989). UV is customarily separated into three wavelengths, UV-A, UV-B and UV-C. UV-C (200-280 nm) is a great degree hurtful to living creatures, yet not significant under natural conditions of solar radioactivity. UV-B (280-320 nm) is specifically noteworthy because although this wavelength characterizes only roughly 1.5% of the total spectrum, it can have a variability of detrimental effects in plants. UV-A (320-400 nm) denotes about 6.3% of the incoming solar radiation and is the minimum lethal part of UV radiation (Hollosy, 2002). Plant reactions to any given dose of radiation are extraordinary, UV-A is less powerful and effective than UV-B and UV-C. Exceedingly lively UV-C (wavelengths 280 nm) is emphatically consumed by oxygen and ozone in the stratosphere with the end goal that none of this cleaning radiation is available in global sunlight. Possibly hurtful UV-B (280–315 nm) is emphatically consumed by barometrical ozone, however around 4% of global radiation is UV-B from 290 to 315 nm. UV-A (315–400 nm) is not weakened by air ozone, and this less harming radiation is a vital photomorphogenic signal in plant development.

The seeds of Pusajwala Chilli are exceedingly nutritious, superb, virtue, freshness, simple to utilize and unequivocally handled. These are hot and abandon from light green to red when developed. The pod appears like finger-shaped, curved and with 4.5 inches long. In differentiating, the seeds of Surajmukhi are also a white in shading and blossom consistently, freshness, and simple to development. These plants require sunshine, the more sunlight the fruits receive the warmer they will develop. It will nurture well in a well-drained in 20-35 $^{o}$C and productive soil. On the off chance that planting in pots make certain to utilize a decent natural fertilizer that will hold dampness. Therefore, Pusajwala Chilli was the only main variety utilized for the present investigation for the experimentation of both pre-and post-mutational examinations.

No comprehensive work related to the AFLP-PCR investigation of the varieties of *C. annum* species with reference to impact of both pre-and post-mutational examinations. Henceforth, the present study on the *C. annum* species by molecular characterization and genetic relationship before and after the exposure of UV radiations was carried out by concentrating mainly on the following goals. The present paper goals to emphasis on (i) study the comparative changes in the biochemical composition because of impacts of the mutation in the varieties of the *Capsicum* species and, (ii) to evaluate the changes in the molecular characterization of the varieties of the *Capsicum* species by using AFLP technique.

## METHODOLOGY

*Conduction of Experiments*

The present investigation was carried out on the evaluation of mutation and its molecular characterization on the *Capsicum* species (Angiosperms: Solanales: Solanaceae) throughout the experiment. At first the seeds were sowed to develop the plants which were chosen for the present investigation.

*Sowing of seeds*

The seeds of *Capsicum* species for the present study were gathered from the Lalbagh Botanical Garden, Bengaluru, India. The dry seeds of *Capsicum* species were sown independently in small plastic pots at Azyme Biosciences Laboratory Private Limited, Jayanagar, Bengaluru 560 069, packed with organic-manure (vermicompost). Consistently showering the water for their development of

*Capsicum* species and the development parameter was estimated utilizing standard estimating tape/scale for each substitute days. Fourteen replicates of two rows were used for normal plant development of *Capsicum* species (see Figure 1a).

*Morphometric analysis of growing plants*

The morphometric examination on seeding and developing plant of *Capsicum* species were done from the day of sowing to the 10th day of the developing plant (see Figure 1b).

*Effect of mutation on Capsicum plant in different time intervals*

The Chilli plant seed was taken and sown in the pits (eight seeds) in the laboratory providing favourable environmental condition for its appropriate development. The growth of the plant of *Capsicum* species was detected every week until the point that it came to 15 cm height. After the developing was seen to the 15 cm, the plant was exposed under UV-C radiations (wavelengths 280 nm) for different time intervals with ten minutes gap, (such as 10 min, 20min, 30 min, 40 min, 50 min and 60 min) (Fig. 1c).


**Fig. 1** a) Sowing seeds and then sprouting of *Capsicum* species after 1-2 days. b) Growing plants of *Capsicum* species after 10 days. c) Grown plants of *Capsicum* species exposed to Ultraviolet-C radiations in UV chambers on the 11th day.

*Preparation of phosphate buffer*

Leaves from plant of *Capsicum* species was taken and weighed up to 1 g. The phosphate buffer was prepared by addition of Potassium dihydrogen orthophosphate (100 mM) and Di-potassium hydrogen phosphate (100 mM) and the pH was set to 7. The leaves were homogenized in 10 ml of phosphate buffer. The mixture was transferred into cleaned centrifuge tubes for each sample and subjected to centrifugation at 10,000 rpm for ten minutes. The supernatant was transferred into clean tubes and refrigerated for the further analyses. The leaves of *Capsicum* species from the Pusajwala variety were utilized as material for DNA extraction.

*Estimation of Biomolecules and Stress Enzymes*

The estimation of biomolecules and the stress enzymes for both the normal Chilli plant and the mutated plant exposed under the UV rays was conducted. The extraction is usually carried out with buffer used for the analysis of enzyme assay. Weigh 1 g of the sample of *Capsicum* species from both the normal and mutated plant with different time intervals and grind well with a pestle and mortar in 5-10 ml of buffer. Then centrifuge it and use the supernatant for the estimation of biomolecules and the stress enzymes.

*Estimation of Biomolecules*

The proteins and carbohydrates were estimated using the methods followed by the Lowry *et al.*, (1951) and Miller`s method (1972), respectively. A stock solution of 100 µg/ml of Bovine Serum Albumin (BSA) was prepared by dissolving 10 mg of BSA in 100ml of distilled water in a standard volumetric flask. Using this stock solution, dilution ranging from 40-200 µg/ml was prepared. To 1 ml of each of the dilutions, 5 ml of the alkaline copper reagent "C" was added and the test tube was

incubated at room temperature for 10 min. To the incubated tubes, 0.5 ml of Folin's Ciocalteu reagent was added and tubes were further incubated in dark for 30 min for reaction to complete. The absorbance of protein samples was read at 660 nm (Lowry *et al.*, 1951). A stock solution of 1 mg/ml was prepared by dissolving 10 mg of glucose in 10 ml of water using a volumetric flask. The stock was further diluted with water to obtain a final sugar concentration of 40 µg/ml to 200 µg/ml. To 1ml dilution, 3 ml of 3, 5 Dinitrosalicylic acid (DNS) was added and the tubes were incubated for 5-8 min in boiling water bath. The solution was later added with 40% of 1 ml sodium potassium tartarate and absorbance of carbohydrate samples was read at 540 nm (Miller, 1972). The absorbance of the protein and carbohydrate samples were plotted on the standard graph and extrapolated to get the concentration of proteins and carbohydrates.

Similarly, dissolve 100 µl of oil or melted fat in 50 ml of the neutral solvent in a 250 ml conical flask. Add a few drops of phenolphthalein indicator. Titrate the contents against 0.1 N potassium hydroxide. Shake constantly until a pink color which persists for 15 seconds is obtained. The lipids were estimated by the Cox and Pearson (1962) and used the following formula:

$$\text{Acid value } \left(\text{mg}\frac{\text{KOH}}{\text{g}}\right) = [(\text{v} - \text{b}) \text{ x Normality of KOH x } 28.2]/ \text{ Weight of Sample (g)}$$

Where V= volume of the sample,
B= blank value,
N= normality of KOH- 0.1 N and
56.1 is the molecular weight of Oleic acid.

*Estimation of Enzyme extract*

Homogenize plant tissue of *Capsicum* species in a blender with M/150 phosphate buffer (assay buffer diluted 10 times) at 1-4 °C and centrifuge stir the sediment with cold phosphate buffer, allow to stand in the cold with occasional shaking and the repeat the extraction once or twice. The extraction should not take longer than 24 h. Use the combined supernatants (sometimes opalescent) for the assay. The enzyme activity can change considerably on storage of the tissue for the comparative studies, therefore, always use the same conditions of extraction, storage and temperature.

The peroxidase and catalase activities were estimated using the method followed by the Malik and Singh (1980), and Luck (1974), respectively. 3 ml of phosphate buffer (0.1M) was added along with 0.05 ml guaiacol (20mM) and 0.03 ml of hydrogen peroxide (0.042 %). To this mixture 0.1 ml of enzyme (sample) was added. The absorbance was recorded at 436 nm. This assay was carried out for each sample and the reading of each sample of different concentration was taken from the 0th minute to the 5th minute (Malik and Singh, 1980). Also, 3 ml of phosphate buffer (0.1 M) was added along with 0.03 ml of hydrogen peroxide (0.042 %). To this mixture, 0.1 ml of enzyme (sample) was added. The absorbance was recorded at 230 nm. This assay was carried out for each sample and the reading of each sample of different concentration was taken from the 0th minute to the 5th minute (Luck, 1974). The activity of peroxidase and catalases in the samples was calculated by the formula mentioned below:

$$\frac{\text{Change in absorbance / min}}{\text{Extension coefficient}} \text{ X } \frac{\text{total volume}}{\text{volume of sample}}$$

*Estimation of Antioxidant activity*

The percentage of antioxidant activity of each substance was assessed by Diphenyl-1-picrylhydrazyl (DPPH) free radical assays and ABTS free radical assays followed by the Brand-Williams *et al.*, (1995) and Witayapan *et al.*, (2007), respectively. DPPH solution was then diluted with methanol to obtain an absorbance of 1 at 571 nm. After the addition of 10 µl, 20 µl, 30 µl, 40 µl

and 50 µl of plant extracts and the volume was made up to 50 µl by addition of methanol. To all the tubes, 3 ml of diluted DPPH solution was added and the absorbance was measured after incubation in dark for 15 min. It reacts with antioxidants compounds, which can donate hydrogen, it is reduced. The changes in colour (from deep violet to light violet) were read (Absorbance read at 571 nm after 100 minutes of reaction) using a UV-Vis Spectrophotometer. The mixture of methanol (10 ml) and reagent (3 ml) serve as blank. The control solution was prepared by mixing ethanol 3.5 ml and DPPH radical solution 0.3 ml (Brand-Williams *et al.*, 1995). The samples were reacted with the stable ABTS radical in a methanol solution. ABTS cation radical was produced by the addition of 7 mM ABTS in 50 ml of water (A) and 2.45 mM potassium persulfate in 50 ml of water (B). Take A and B solution in 1:1 ratio and incubate in a dark room before use for 12-16 hours at room temperature. ABTS solution was then diluted with methanol to obtain an absorbance of 1 at 734 nm. After the addition of 10 µl, 20 µl, 30 µl, 40 µl and 50 µl of plant extracts and the volume was made up to 50 µl by addition of methanol. To all the tubes, 3 ml of diluted ABTS solution was added and the absorbance was measured after incubation in dark for 30 min. It reacts with antioxidants compounds, which can donate hydrogen, it is reduced. Blank was also prepared by addition of methanol (10 ml) and the reagent (3 ml) (Witayapan *et al.*, 2007). The radical scavenging activities was calculated as $IC_{50}$ value (Vats, 2012). Percent inhibition of absorbance at 734 nm was calculated using the formula:

$$\text{Percentage inhibition} = \frac{1 - O.D}{1} \text{ X } 100$$

*Molecular Characterization by using Amplified fragment length polymorphism (AFLP)*

*Isolation of DNA (Total genomic DNA) by Cetyl trimethylammonium bromide (CTAB) Method*

Take 1 g of leaf of *Capsicum* species, grind it by adding 15 ml of extraction buffer by using mortar and pestle. Transfer the centrifuge tubes and incubate at 60-65 °C for 1 h. Allow at room temperature and add 5 ml of 24:1 chloroform isoamyl alcohol and gently vortex. Centrifuge at 10,000 rpm for 10 min. Take supernatant and from that solution, take 1/10th volume of % M NaCl. Add equal volume of ice-cold propanol. Incubate for overnight. Centrifuge at 12,000 rpm at 4 °C for 10 minutes. Take pellet, dry and dissolve in 0.5 ml of TE buffer. Agarose Gel Electrophoresis was done by the pouring, running and imaging of the gel using the standard protocol.

*Purification of DNA*

Take 0.3 ml crude DNA sample in separate Eppendorf tubes and add equal volume of phenol: chloroform: isoamyl alcohol mixture (ratio – 25:24:1). The above mixture is vortexed and centrifuged at 10,000 rpm for 10 minutes at 4 °C. Carefully pipette out aqueous layer (top most layer) into another Eppendorf and add equal volume of 24:1 chloroform: isoamyl alcohol mixture. Gently vortex the mixture and centrifuge at 10,000 rpm for 10 minutes at 4° C. Again, transfer the aqueous layer carefully to another Eppendorf tube and add double the volume of chilled ethanol and incubate the sample mixture for 30 minutes in ice cold condition (4 ºC). Centrifuge at 10,000 rpm for 20 minutes at 4° C. The pellet obtained is air dried to remove excess ethanol and dissolve in 0.1 ml of TE buffer and stored at 4 ºC until future use. Agarose gel electrophoresis is once performed again.

*Amplified fragment length polymorphism (AFLP) Analysis*

10xTBE -Tris Base 121 g, Boric Acid 51.3 g, EDTA 3.7g (80ml 0.25M EDTA pH 8), Distilled water to 1 litre, Heat sterilized and stored at room temperature. Acrylamide Mix 4.5%. Urea - 420g.10x TBE-100 ml. Acrylamide mix 40%- 115 ml. Distilled water to 1 litre. Stored at 4ºC as this must be used from chilled, T10E0.1 pH 8.0, 10mM Tris pH 8, 0,1mM EDTA, pH 8, Stored at room temperature, Acrylamide gel loading dye, 98% Formamide, 0.025% Bromophenol blue, 0.025% Xylene cyanol, 10mM EDTA, pH 8 (Stored at room temperature).

AFLP analysis was done by template preparation using forward and reverse primers, double digestion using a pair of restriction enzymes which is *Eco*RI and MSE I, and ligation of adapters using the standard protocol. Then, amplification of AFLP templates was done by the preamplification and re-amplification using the standard protocol. After PCR amplified product was run agarose gel electrophoresis.

Moreover, Euclidean distances method of cluster analysis of *Capsicum* species was carried out to create a dendrogram to assess the scoring of DNA bands from UV-C radiations exposed and unexposed plants in six different time intervals using PAST version 1.60 software (Hammer *et al.*, 2001).

## RESULTS AND DISCUSSION

*Analysis of Growth Parameters*

The seed sample of *Capsicum* species were collected from the "Lalbagh Botanical Garden'', Bengaluru city, India. The seeds of *Capsicum* species were collected in a container and taken to the laboratory for further studies (see Fig. 1a). The plants of *Capsicum* species were grown for several (maximum – four to five) weeks in controlled environment room and also in vermiculite medium using pots.

The measurement of growth parameters was done on the plants which are grown in the small pits. The plants were transfer to other pits to study the uniform growth of the plants. The parameters such as plant height, leaf width and number of leaflets per plant were observed that the growth of all plants was uniform (Fig. 1b) and the same plants were exposed to UV radiations in UV chambers at different time intervals (Fig. 1c).

*Estimation of Biomolecules and Stress Enzymes*

*Estimation of Protein*

The amount of protein in control was calculated on the basis of the standard graph ($y = 0.0017x + 0.0118$; $R^2 = 0.9921$) obtained in *Capsicum* species. Similarly, the least protein content was found in 60 min mutated plant and had 799.997 μg/ml of protein that showed decrease contents compared to the other plants (Fig. 2a). This might be because of damage existed in peptide bonds formed between the α-amino group of one amino acid and the α-carboxyl group of other amino acid corrosive that avoided in yielding a dipeptide and then polypeptide. Thus, polypeptide chain unfurls from various conformations in the protein giving distinctive basic structures.

*Estimation of Carbohydrates*

The amount of carbohydrate in control was calculated on the basis of the standard graph ($y = 0.0404x + 0.0142$; $R^2 = 0.9847$) obtained in *Capsicum* species. Similarly, the least carbohydrate content was found in 60 min mutated plant and had 39.13 μg/ml of carbohydrate that showed decrease contents compared to the other plants (Fig. 2b). This might be because of harm existed in both straight and spread polymer of homopolysaccharides f framed inside the layer bound cell organelles that counteracted in keeping up trademark appearance and shape. Additionally, harms happened in cellulose by detaching together by β (1-β) linkages. These chains are straightforwardly separated to each other by intermolecular and intramolecular hydrogen bonds and Vander Waals force.

*Estimation of Lipids*

Lipids or free fatty acids activity was calculated by the formula given for each of the sample in the *Capsicum* species. Minimum free fatty acid percentage was seen in 673.2 μg/ml concentration in

60 min post mutated samples. Hence, the present study revealed that 60 min of the post mutated samples is required for normal growth of the plant with the minimum lipid content (Fig. 2c). This might be because of destruction existed in covalent bonds formed in the long-chain saturated and unsaturated fatty acids that capacities as damage to plant from water gain and from rough harm. Along these lines long chain unfurls from various compliances in the lipids giving distinctive straightforward structures.

*Estimation of Enzyme extract*

*Estimation of Stress Enzymes by Peroxidase activity*

The highest percentage of inhibition by the peroxidase activity (0.1685 µg/ml) was found in 50 µl mutated plants of *Capsicum* species that revealed increase activity compared to the other samples (Fig. 2d). In this manner, Ultraviolet-C radiation initiated oxidative worry in Chilli by expanding lipid peroxidation and layer porousness which demonstrates that points of confinement of resistance are substantially less than wounded caused by UV radiation (Mahdavian *et al.*, 2008). In this way, the peroxidase activity is additionally a vital part of the antioxidant stress system for scavenging H2O2. However, catalase changes $H_2O_2$ into $O_2$, while peroxidase breaks down $H_2O_2$ by the oxidation of co-substances (Gaspar *et al.*, 1991).

*Estimation of Stress Enzymes by Catalase activity*

The highest percentage of inhibition by the catalase activity (0.078 µg/ml) was found in 60 µl mutated plants of *Capsicum* species that showed increased activity compared to the other samples (Figure 2e). Oxidative stress is supplemented by the amalgamation of hydrogen peroxide, which is typically detoxified by catalase activity in the peroxisomes and by ascorbate peroxidase in the cytosol, mitochondria, and chloroplasts of the *Capsicum* species (Asada, 1999).

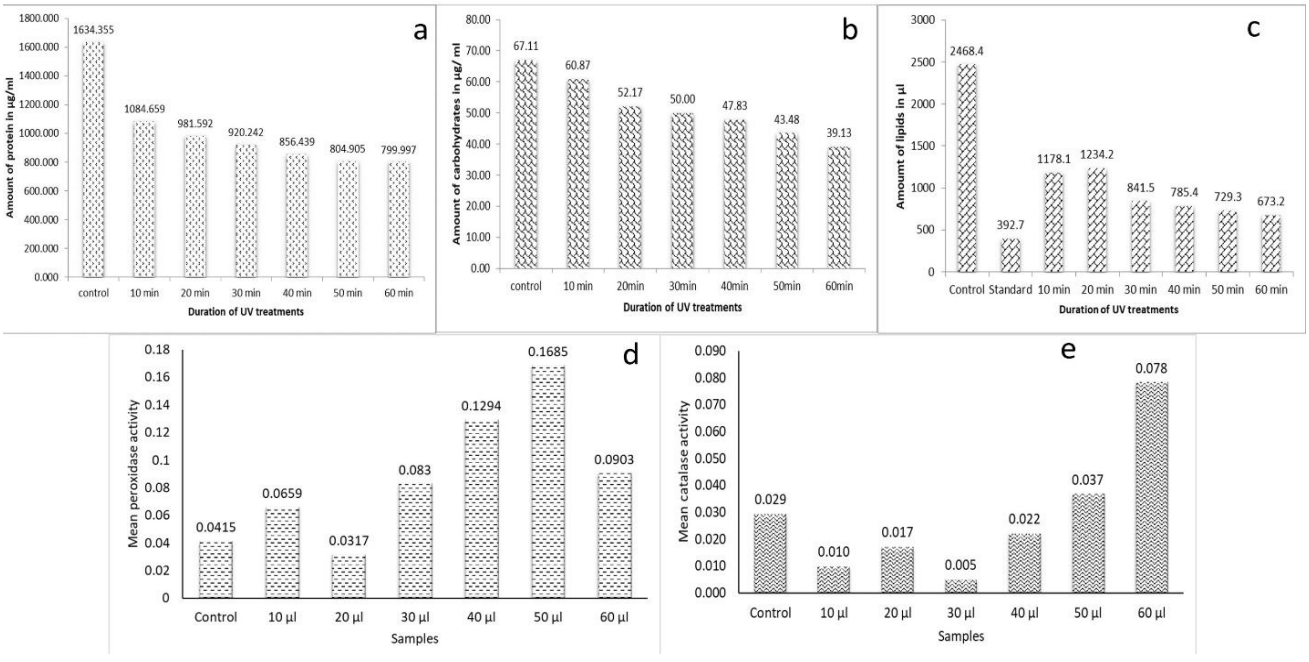


**Figure 2.** Effect of UV-C treatment on the a) protein b) carbohydrate and c) lipid contents, d) peroxidase and e) catalase activities in the normal and mutated plants of *Capsicum* species

*Estimation of Antioxidant activity*

*Estimation of free radical scavenging activity of Diphenyl-1-picrylhydrazyl (DPPH) assay*

The highest percentage of inhibition by the DPPH activity (79.44±1.61 µg/ml) was found in 40 µl mutated plants of *Capsicum* species that indicated increased activity compared to the other samples (Figure 3a). After UV exposure, the capacity of marginally higher DPPH radical scavenging capacity

was found in UV-C treated plants. UV-C treatments did not cause checked modifications in DPPH radical scavenging capacity. UV-C treated peppers kept up somewhat higher DPPH radical scavenging capacity mainly when the appearance of the disorder was already advanced. In this way, the UV-C treatment expanded aggregate cell antioxidants (Costa *et al.,* 2006; González-Aguilar *et al.,* 2007).

Likewise, a decrease in biochemical synthesis of the peppers treated with a UV-C radiation was accounted for by Vicente *et al.* (2005). UV-C treatments diminished the occurrence of Chilling damage and seriousness. UV-C treated leaves also exhibited lower electrolyte discharge, respiration rate and phenolic compound substance recommending lower injury in response to low temperature stockpiling. The UV-C treatments could be a valuable method for decreasing decay and maintaining bell pepper fruit quality. Moreover, Chilling damage occurrence and seriousness could be decreased by short UV-C treatments. They initially evaluated if a higher dose might be even more beneficial. On analyzing the DPPH radical scavenging capacity, immediately after UV-C treatment, distinctions were recognized between control and treated plants (Vicente *et al.,* 2005).

*Estimation of free radical scavenging activity of 2, 2′- azino-bis (3-ethylbenzoithioazoline-6-sulphonic acid) (ABTS) assay*

The highest percentage of inhibition by the ABTS activity (97.02±0.45 µg/ml) was found in 50 µl mutated plants of *Capsicum* species that exhibited increased activity compared to the normal plants (Figure 3b). Enzymes for example, polyphenol oxidase, ascorbate peroxidase, and glutathione reductase in addition to the peroxidase and catalase demonstrated upgraded action in UV-C treated plants and these enzymes proteins may fill in acclimatization systems to scavenge the harmful free radicals of oxygen created under pressure condition. The results of the present work illustrate that in *Capsicum* species, UV-C radiation generates antioxidant substances that provide protection against UV-C radiation. Along these lines it very well may be presumed that UV radiation induced both enzymatic and nonenzymatic activities that secure plants against UV radiation in a given dose in *Capsicum* plant species.

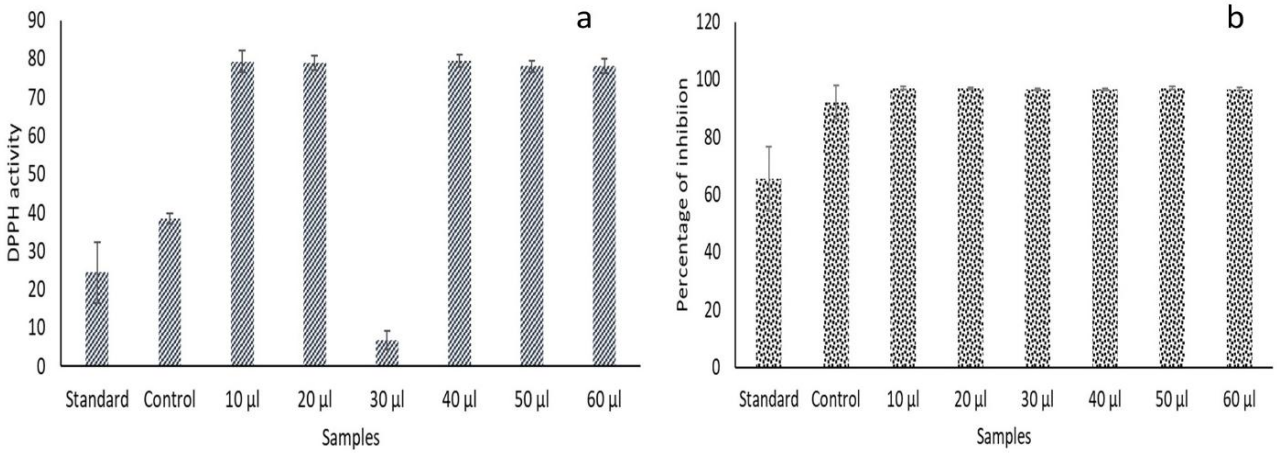


**Figure 3.** Effect of UV-C treatment on the a) DPPH and b) ABTS activities in the normal and mutated plants of *Capsicum* species

Besides, Ultraviolet-C radiation is a possibly harmful, physical mutagenic agent and shape an important component of terrestrial radiation to which plants have been uncovered since attacking area (Mahdavian *et al.,* 2008). From that point forward, plants have advanced components to maintain a strategic distance from and fix from UV radiation injury. Most of the compounds accumulated are directly involved in UV-C protection; they are either efficient in filtering excess

radiation or in scavenging radicals. The substance of chlorophyll a, b and carotenoids of pepper leaves were decreased significantly in those plants which were presented to UV-C radiation and contrasted with control plants. Thus, Ultraviolet-C radiation induced oxidative stress in Chilli by increasing lipid peroxidation and membrane permeability which indicates that limits of tolerance are much less than damaged caused by UV-C radiation (Mahdavian *et al.*, 2008). Likewise, the impact of UV-C radiation on chlorophyll, flavonoids, anthocyanin, proline, membrane permeability, lipid peroxidation and UV-absorbing compound was analyzed with reference to the *Capsicum* species. The seeds were treated with different concentration of gamma rays and the effect of gamma rays on phytochemical constituents in Chilli was considered on M2 generation. The phytochemical constituents induced chlorophyll, Capsaicin, Oleoresin, Capsanthin and Ascorbic acid (Aruldoss and Mullainathan, 2015).

The impacts on the structure and ultrastructure of Chilli plants when presented to UV radiation under greenhouse conditions. Changes in shoot development demonstrated the critical decline in UV-C exposed plants. Leaf area also diminished altogether in UV-C-exposed plants. Stomata expanded in number and size in UV-C exposed plants. Chloroplast thylakoids were enlarged and starch decrease was seen at the ultra-basic dimension. UV treatment resulted in the formation of crystals in the peroxisomes of mesophyll cells. The development of these crystals was due to an increase in catalase activity, which is an antioxidant enzyme. The Chilli plants were delicate to UV and the discoveries gave the knowledge into the physiological changes during UV exposure, and showed this plant was progressively touchy to UV-C radiation (Sarghein *et al.*, 2011).

*Molecular Characterization by using Amplified fragment length polymorphism (AFLP)*

In AFLP strategy, a total genomic DNA is processed with two restriction enzymes and adaptors of known sequence are then ligated to the DNA fragments. Primers complementary to the adaptors, with extra 1-3 selective nucleotides on the 3' end are utilized to intensify the restriction fragments. The PCR amplified parts would then be able to be isolated by gel electrophoresis and the banding patterns visualized. AFLP profiles require no earlier DNA sequence information and the number and nature of amplified fragments are adjusted by the decision of primer pair.

The DNA of *Capsicum* species is isolated from cells in stepwise manner. DNA was released from the cells and nucleus (Figure 4a). All the chemicals and buffers aid for the release and purification of DNA. After extraction of pure DNA from samples, DNA is quantified using agarose gel electrophoresis and the DNA fragments/ bands are observed under UV light (Figure 4b). The purified DNA fragments are double digestion using the enzyme ECORI and MSEI and the bands are observed under UV light (Figure 4c).

This enzymatic can be used for cleaving DNA molecule at specific sites, ensuring that all DNA fragments that contain a particular sequence at a particular location have same size, furthermore each fragment that contain the desired sequence has the sequence located at exactly the same position within the fragment it). The resulting digested DNA is very selectively amplified using PCR reamplification (Figure 4d).

A dendrogram showing similarity in the DNA bands of *Capsicum* species based on the scoring of unexposed and exposed plants in six different time intervals of UV-C radiations with three major clusters showed significant negative affinities. 50-60 minutes of UV-C radiations exposed plants accounted for low scoring of DNA bands belonging to the first cluster; 10 and 40 minutes of UV-C radiations exposed plants of DNA bands in addition to the 20-30 minutes of UV-C radiations exposed plants of DNA bands accounted for moderate scoring in the second cluster, whereas normal

plants without exposure of UV-C radiations accounted alone for the third cluster with a high scoring of DNA bands (Figure 4e).

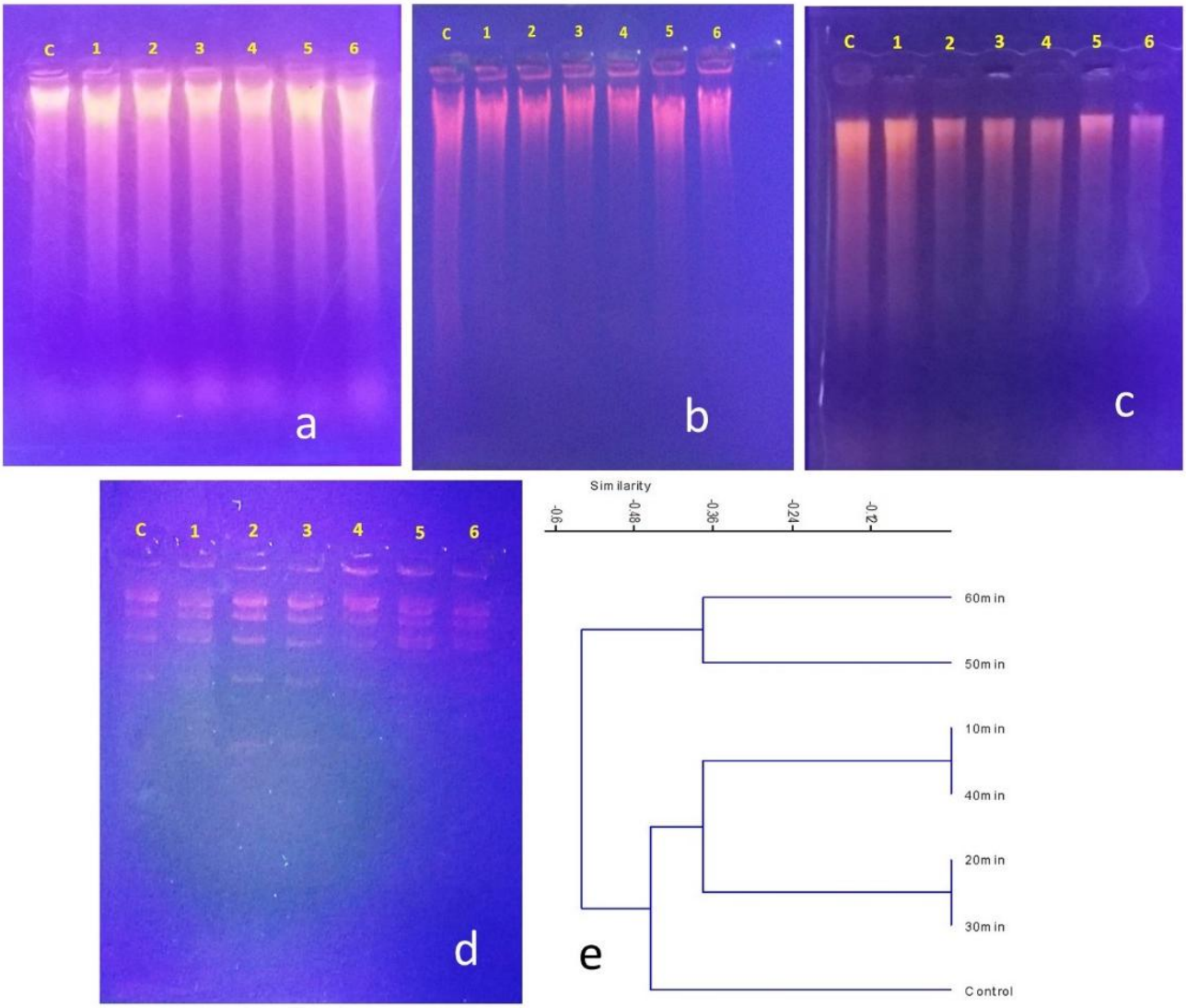


**Figure 4.** a) DNA isolation bands and b) DNA purification bands of *Capsicum* species from different time intervals of UV exposure using CTAB methods. c) Purified DNA bands and d) Reamplification DNA bands of *Capsicum* species obtained from different time intervals of UV exposure using double digestion methods. Lane C is DNA of the control plant without UV exposure, Lanes 1, 2, 3, 4, 5 and 6 indicates 10, 20, 30, 40, 50 and 60 min after UV rays exposure respectively. e) Cluster analysis of DNA bands of *Capsicum* species obtained from scoring of UV-C radiations exposed and unexposed plants in different time intervals

Henceforth, there is a little variety in the occurrence of DNA bands of *Capsicum* plant mutated at 50-60 minutes intervals after the UV-C radiation exposure when contrasted with the other plant mutated samples. Our outcomes uncovered that the perception of incited UV-C mutation in of *Capsicum* species was maybe recorded by these divided DNA banding designs. Still more than one hour of UV exposure is required to make/create the fake change in the chose plant source.

DNA from plants presented to UV radiation displayed polymorphic bands which were not detectable in DNA of unexposed or controlled plants of *Capsicum* species. The upgraded development of AFLP polymorphisms was additionally seen in DNA of plant presented in situ to a physical source for mutagenesis. The correlation among "unexposed" and "exposed" genomes

demonstrate that AFLP analysis can be utilized to assess how the mutagenic agents change the structure of DNA in living life forms.

The AFLP procedure has the advantage of sampling many loci simultaneously and it is more robust than discretionary preparing strategies, for example, RAPD, in light of the fact that increasingly stringent conditions are utilized. Along these lines, AFLP gives a novel and amazing asset for gene tagging technique of any origin or complexity (Blears *et al.*, 1998). It is ordinarily acquired in Mendelian form and in this way be utilized for typing, identification of molecular marker and mapping of genetic loci.

The present study privileges an induced mutation by Ultraviolet-radiation and its impact on the plant cells and DNA structure of Chilli plants (Figure 5) utilizing the physiological methods and AFLP investigations.

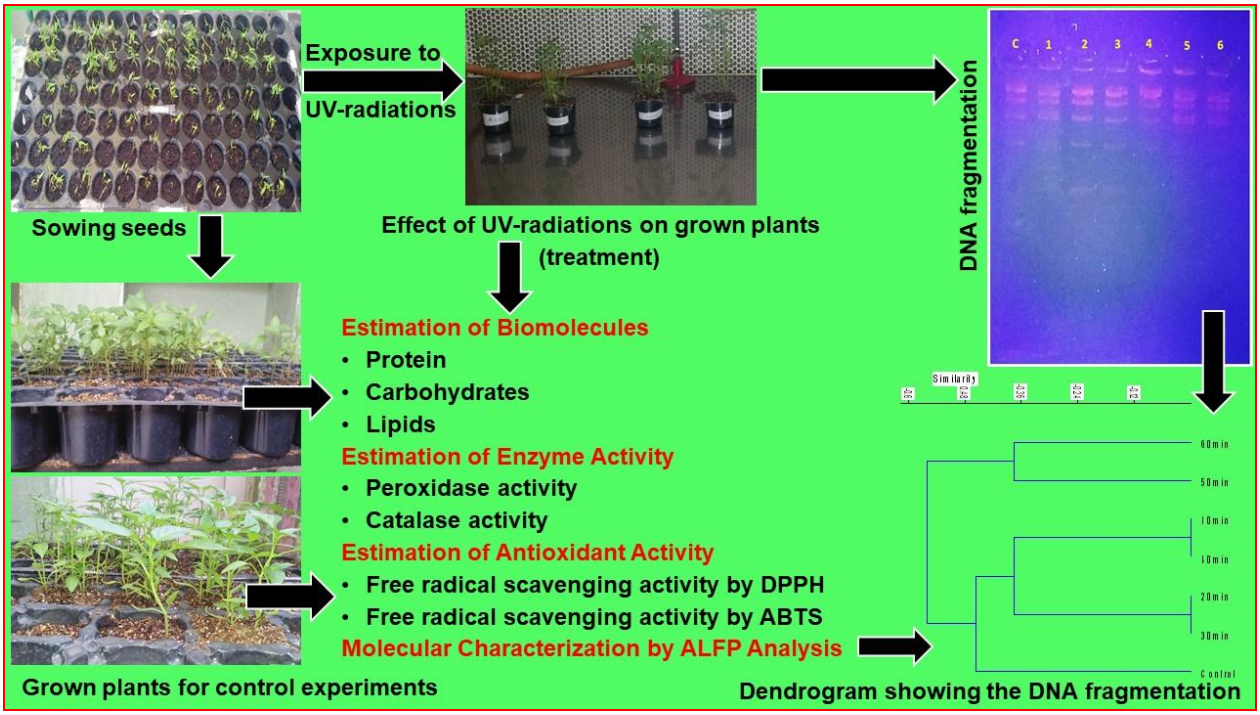


**Figure 5.** Summary of the findings

## CONCLUSION

The present study on the *Capsicum* species affords the information to understand the molecular changes and genetic relationship for the experimentation of both pre-and post-mutational examinations from the acquaintance of UV radiations. Our results indicated the presence of a positive relationship between the biochemical changes and the molecular characterization changes because of the impact of mutation, anyway further examinations are expected to illustrate this association at the molecular level in *Capsicum* species. The application of UV-C radiations as mutation sources for valuable instigated mutation for the discovery of biochemical and molecular changes in these experiments.

Moreover, the present examination proposes that the AFLP test connected related to different biomarkers from larger amounts of natural association would demonstrate an amazing eco-toxicological device. This should be possible at the molecular level by normal DNA sequencing in which pre- and post- mutational changes can be distinguished utilizing Real-time PCR examination. Now most advanced next generation sequencing and genotyping technologies have also been

creating more genomic assets in the *Capsicum* species which must be utilized proficiently for product enhancement in future.

## ACKNOWLEDGEMENTS

We thank Dr. M. Mahesh, CEO of Azyme Biosciences Private Limited, 9th Block, Jayanagar, Bengaluru 560 056 for the conduct of desired experiments and providing the laboratory facilities for this work.

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