

## Influence of Integrated Approach of *Azotobacter* and Nitrogen Fertilizer on Various Morpho-Physiological And Biochemical Parameters of *Brassica oleracea* L. var. capitata

PEER SAFFEULLAH<sup>1\*</sup>, NEELOFER NABI<sup>2</sup>, and SHAHID UMAR<sup>1</sup>

<sup>1</sup>Department of Botany, Jamia Hamdard, New Delhi, India.

<sup>2</sup>Department of Botany, University of Kashmir, Srinagar, India.

### Abstract

This study was formulated to evaluate the impact of bacterization with *Azotobacter* of two cabbage genotypes (Pusa Early golden acre and Pusa drum head) under field conditions. The study was performed in herbal garden of Jamia Hamdard, New Delhi. The plants were treated with graded treatments (60,120,180 kg N ha<sup>-1</sup>) of N alone and in combination with seedling inoculation with *Azotobacter*. The plants were sampled and tested for various morpho-physiological and biochemical parameters. Chlorophyll content, NR activity, protein content, sugar content and phenol content was found to be significantly higher in plants treated with N in combination in *Azotobacter*. Thus, the use of *Azotobacter* as a supplement or biofertilizer in integrated nutrient management systems was highly recommended to minimize the application rates of synthetic fertilizers and attain the goal of sustainable agriculture.



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### Keywords

*Azotobacter*;  
Cabbage;  
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### Introduction

Food security is one of the critical challenges we are facing today.<sup>1</sup> Nevertheless, agricultural lands have been over exploited for centuries; and its fall outs are soil degradation, erosion, and increased greenhouse gas emissions, due to higher dependency on agronomic inputs.<sup>2</sup> Some of the proposed options for improving soil fertility include organic amendments, no-till farming, use of bio-fertilizers and integrated nutrient management system.<sup>3</sup> World agricultural output has tremendously increased since 1950's


by the effort of scientific community to ensure food security. But these modern methods of agriculture have led to deleterious effects on our environment.

About 80-90 million tons (MT) of nitrogen (N) fertilizers are supplied to soil throughout the world each year.<sup>4</sup> To maintain the sustainability of agricultural production is itself a great challenge. However during the last few years, bio-fertilizers have appeared as an imperative part of integrated nutrient management system and seems a promising

**CONTACT** Peer Saffeullah ✉ psaffeullah.139@gmail.com 📍 Department of Botany, Jamia Hamdard, New Delhi, India.



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approach for increasing crop yield and free nutrient supply to soil.<sup>5</sup> In recent years, there has been abundant attention in establishing associations among crops and various N<sub>2</sub>-fixing microbes.<sup>6</sup> *Azotobacter* (AzB) can be a crucial alternate of synthetic N fertilizers because it delivers N in the form of ammonia (NH<sub>3</sub>), nitrate (NO<sub>3</sub><sup>-</sup>) and amino acids, which could be potential replacements of inorganic nitrogen source.<sup>7</sup> It also aids to sustain the plant growth and yield even in situation of low phosphate content in soil, as well as assists in uptake of nutrients which enables improved utilization of plant root exudates.<sup>8</sup>

The genus *Azotobacter*, belonging to family *Azotobacteriaceae*, is the key group of heterotrophic non-symbiotic N-fixing bacteria. The non-symbiotic free living *Azotobacter* is mostly related with N fixation in plant rhizosphere<sup>9</sup> and is capable of fixing at least 10 mg N per g of carbohydrate.<sup>10,11</sup> *Azotobacter* spp. are most specifically noted for their N fixing ability; although they have also been noted for their capability to provide different growth hormones (like IAA, gibberellins and cytokinins), vitamins and siderophores.<sup>12</sup> When *Azotobacter* is applied to seeds, seed germination is enhanced to a substantial extent besides improving growth.<sup>13</sup>

*Brassica oleracea* L.var.capitata, a member of the genus *Brassica* (family *Brassicaceae*), commonly known as cabbage, is consumed either raw as salad or processed in different ways such as boiled or fermented.<sup>14</sup> Cabbage has extensive use in traditional medicine due to its antioxidant, antibacterial and anti-inflammatory properties.<sup>15</sup> It is also used in easing of symptoms linked with numerous gastrointestinal disorders as well as in treatment of minor cuts and wounds and mastitis.<sup>15</sup> The seeds are anthelmintic, diuretic, laxative and stomachic. They have been used in the treatment of gout.<sup>16</sup> The phytochemicals present in cabbage can alleviate and stabilize the body's antioxidant and detoxification mechanisms that eradicate cancer-causing substance.<sup>17,18</sup> Since the indiscriminate and excessive use of N fertilizers is often practiced in cabbage farms, in pursuit of maximum yield. So this study was designed to test this alternative fertilizer (*Azotobacter*) to lower the use of synthetic N. Keeping in view the importance of cabbage, the

present study was conducted to elucidate the effect of N and AzB treatments on cabbage (*Brassica oleracea* L. var. capitata).

### Materials and Methods

Authentic seeds of *Brassica oleracea* L. var. capitata [genotype Early golden acre (L) and Pusa drumhead (H)] were procured from IARI, New Delhi. Seeds were surface sterilized by 0.1% mercuric chloride solution for 3 min, then washed with sterilized distilled water (5-times) and air-dried at an ambient temperature of 27°C in the laboratory. Following treatments of N (supplied as urea) and AzB were applied at the time of sapling transplantation as seedling treatment after 3-4 weeks of seed germination (Table 1).

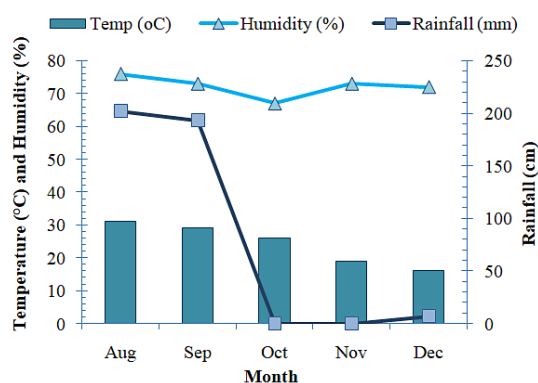
**Table 1: Various treatments of N and *Azotobacter* (AzB) (alone and in combination) applied to low and high NE genotypes used in the present study**

Name assigned	Treatment applied
T <sub>0</sub>	Control
T <sub>1</sub>	60 kg ha <sup>-1</sup> N
T <sub>2</sub>	60 kg ha <sup>-1</sup> N + AzB
T <sub>3</sub>	120 kg ha <sup>-1</sup> N
T <sub>4</sub>	120 kg ha <sup>-1</sup> N + AzB
T <sub>5</sub>	180 kg ha <sup>-1</sup> N
T <sub>6</sub>	180 kg ha <sup>-1</sup> N + AzB
T <sub>7</sub>	AzB alone

The saplings were dipped in liquid containing *Azotobacter chroococcum* (AzB:DW-1:5) before transplantation. The experiment was performed under natural field conditions in prepared beds in herbal garden of Jamia Hamdard, New Delhi, India. There were 8 beds comprising 3 replicates for each treatment and each genotype. Experiments were arranged in randomized block design with three replicates. The soil is classified as Fluvisol Cambisol (Humic) according to the classification of the Food and Agriculture Organization (FAO Rome). Some of the soil characteristics and climatic conditions of the experimental area are given in Table 2 and Fig. 1, respectively. Plants were sampled at three growth stages and harvested after 90 days of treatment and the results were recorded during the time frame of experiments.

**Table 2: Some physico-chemical characteristics of pre-treated soil (0-30 cm), of the study area. Data represents mean±standard deviations (n=3)**

Soil Characteristic	Value	Unit
pH	7.21±0.4	-
Electrical Conductivity	0.15±0.06	dS m <sup>-1</sup>
Soil organic carbon	1.62±0.2	g kg <sup>-1</sup>
Cation exchange capacity	20.1±0.9	cmol kg <sup>-1</sup>
Available N	0.43±0.04	g kg <sup>-1</sup>
Available P	0.07±0.01	g kg <sup>-1</sup>
Available K	0.26±0.08	g kg <sup>-1</sup>
C/N ratio	3.76±0.12	-



**Fig. 1: Some climatic conditions of the study area during the growing season (August 2018 to December 2018)**

### Parameters Studied

#### Growth Characteristics

Plants were dig up cautiously and cleaned by distilled water. At 12-week stage, plant and root length was noted down. Shoot fresh weight (g) was measured by electronic balance. Plant samples were dried in an oven at 75°C for 3 days. After three days, shoot and root dry weight (g) was recorded again. Dry weights of the entire plant, shoot and root were determined by drying in an oven at 68°C to a constant weight. The number of leaves was manually calculated at the end of the study; leaf area was also measured.

#### Estimation of Total Chlorophyll

Photosynthetic pigments, the chlorophyll a, b and total chlorophyll contents in the leaf were estimated in fresh samples by the method of Hiscox and Israelstam<sup>19</sup> using dimethyl sulphoxide (DMSO).

Vials containing 100 mg of chopped leaves in 10 mL DMSO were covered with aluminum foil and kept in oven at 65°C for an hour. The reaction mixture was taken in a cuvette and finally absorbance was read at 480, 510, 645, 663 nm by using UV-Vis spectrophotometer (Systronics, India).

#### Nitrate Reductase (NR) Activity

Nitrate reductase (NR) activity was assessed by the intact tissue assay method of Jaworsk<sup>20</sup> which takes into account the reduction of NO<sub>3</sub><sup>-</sup> to nitrite (NO<sub>2</sub><sup>-</sup>). Leaf material (250 mg) was suspended in screw capped vial containing 2.5 mL each of phosphate buffer (0.1 M, pH 7.5), potassium nitrate solution (0.2 M) and iso-propanol (5%), and 2 drops of chloramphenicol (0.5%). The vials were incubated at 30°C in the dark for about 2 hours. NR activity in the medium was determined by taking 0.4 mL of incubated solution and 0.3 mL each of sulphanilamide (1% in 3N HCl) and NEDD (0.02%). After 20 minutes, the solution was diluted with 4 mL of distilled water to make the final volume upto 5 mL and its optical density measured at 540 nm. A standard curve was plotted using varying concentrations of potassium nitrite and used for calculations.

#### Total Soluble Protein Content

The total soluble protein content of different sample was estimated following the method of Bradford.<sup>21</sup> Chopped fresh leaf tissues (0.5 g) were crushed in 1.5 mL of phosphate buffer (0.1 M) at 4°C. The homogenate was centrifuged at 5000 rpm for 20 min at 4°C. An identical volume of 10% TCA (pre-chilled) was added to 0.5 mL of the supernatant, which was centrifuged again at 3500 rpm for 20 min. Further, 0.2 mL aliquot was added to 1 mL of Bradford's reagent. The absorbance was noted at 595 nm on a Beckman spectrophotometer (DU 640, Fullerton, USA). The protein content was expressed in mg g<sup>-1</sup>f.w.

#### Estimation of Sugar

This method given by Dey<sup>22</sup> was used for estimating sugar content. 0.1 g of fresh sample was weighted to which 10 ml of ethanol was added and the mixture was incubated at 60°C for 1 hour. After that, 1 mL of phenol was added to 1 mL of aliquot and was vortexed. Then, 5 mL of sulphuric acid was added to the reaction mixture which was later cooled in air. Absorbance was measured at 485 nm.

### Statistical Analysis

Data represents mean±standard deviation (SD). Values are means of three replicates and the presented mean values were analyzed by SPSS software (version 22) for one-way ANOVA. Significant differences were estimated using Duncan's Multiple Range Test (DMRT) at  $p \leq 0.05$ .

### Results

#### Growth Attributes

The plant height, number of leaves and leaf area showed significant variations between the genotypes and different treatments. The variations in the morphological features of two genotypes are given in Table 3.

A significant difference was found in shoot fresh and dry weight at varying N concentrations. The shoot fresh weight was found to increase significantly with an upsurge in N fertilization and was maximum

at  $180 \text{ kg ha}^{-1}$ . It was also found to decrease with the increasing age of the plant. So, fresh weight decreased in all the treatments at post-flowering stages (Table 4). This rise in shoot fresh weight may be attributed to the ability of the plant to assimilate the given nitrogen into proteins and other necessary metabolites. The fall in shoot fresh weight with increasing age may be due to the transition of the plant from vegetative phase to flowering phase of its life cycle, whereby a plant tries to concentrate and protect its progeny rather than accumulating biomass.

A similar trend was followed by the root fresh weight. As we increased the dosage of N the fresh weight was found to increase significantly. The increasing root fresh weight is an evidence to the fact that as plant is supplied more N, it tries to develop its root systems extensively in order to absorb more and more nutrients especially N.

**Table 3: Changes in morphological characteristics of two genotypes of *Brassica oleracea* L. var. as affected by varying applications of N and AzB at harvest stage**

Treatment	Plant height (cm)	Leaf number	Leaf area (cm <sup>2</sup> )
Control L	8.6±0.36e <sup>a</sup>	5±0.82c	34.4±2.36e
Control H	11.2±1.21 D	7±0.91 D	42.32±2.03 F
60 N <sup>a</sup> L <sup>b</sup>	12.66±0.35 d	6±0.39 c	51.02±3.11 d
60 N H <sup>c</sup>	13.26±0.52 C	9±0.65 C	54.06±2.36 E
60 N + AzB <sup>d</sup> L	8.9±0.84 e	7±0.81 b	81.6±3.08 c
60 N + AzBH	13.6±0.65 C	9±0.36 C	56.18±3.26 E
120 N L	14.2±0.39 c	7±0.85 b	117.3±3.44 b
120 N H	18.2±0.82 B	11±0.32 B	76.22±4.11 D
120 N + AzBL	14.6±0.84 c	8±0.25 b	95.12±4.21 c
120 N + AzBH	18.9±0.91 B	10±0.44 B	88.16±4.05 C
180 N L	16.5±0.92 b	7±0.36 b	81.34±3.85 c
180 N H	21.0±0.66 A	12±0.32 A	133.32±3.66 B
180 N + AzBL	17.2±0.45 a	10±0.14 a	102.85±3.25 a
180 N + AzBH	23.4±0.54 A	13±0.35 A	216.25±4.85 A
AzB alone L	13.1±0.36 d	7±0.22 b	83.43±8.22 c
AzB alone H	21.2±0.25 A	8±0.35 C	149.34±5.26 B

<sup>a</sup>N supplied as  $\text{kg ha}^{-1}$

<sup>b</sup>L – genotype low (Early golden acre)

<sup>c</sup>H – genotype high (Pusa drumhead)

<sup>d</sup>AzB- *Azotobacter*

<sup>e</sup>Different small case and uppercase letters denote significant difference ( $p \leq 0.05$  by DMRT) between the treatments in G-low and G-high, respectively

**Table 4: Changes in fresh and dry weight content of root and shoot two genotypes of *Brassica oleracea* L. var. capitata as affected by varying applications of N and AzB at harvest stage**

Genotype x Treatment	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
Control L	7.38±0.66d <sup>e</sup>	0.114±0.003e	178.20±3.99e	23.124±0.108c
Control H	6.53±0.83 C	0.265±0.007 D	348.90±7.51 E	95.139±0.079 D
60 N <sup>a</sup> L <sup>b</sup>	11.80±0.87 b	0.150±0.013 d	538.50±5.48 d	82.020±0.195 b
60 N H <sup>c</sup>	8.00±0.36 A	0.451±0.037 C	1162.83±9.42 D	123.141±0.109 C
60 N + AzB <sup>d</sup> L	9.10±0.62 c	0.170±0.005 c	735.67±5.44 c	85.204±0.114 b
60 N + AzB H	6.30±0.50 B	0.458±0.026 C	1362.47±3.91 C	126.272±0.138 C
120 N L	9.60±0.44 c	0.181±0.005 c	834.20±9.33 b	94.133±0.101 b
120 N H	7.77±0.74 B	0.714±0.021 B	1742.33±11.91 B	142.184±0.116 B
120 N + AzB L	11.80±0.85 b	0.190±0.004 c	882.50±4.14 b	93.336±0.076 b
120 N + AzB H	9.17±0.87 A	0.724±0.011 B	1823.50±12.73 B	158.206±0.035 B
180 N L	8.33±1.17 c	0.515±0.004 a	957.33±8.04 a	121.166±0.149 a
180 N H	10.87±1.55 A	0.942±0.029 A	1982.80±13.91 B	188.483±0.707 A
180 N + AzB L	14.23±1.83 a	0.541±0.015 a	1033.53±4.28 a	127.396±0.609 a
180 N + AzB H	9.87±0.70 A	0.948±0.016 A	2302.40±8.31 A	198.398±0.118 A
AzB alone L	11.77±0.76 b	0.218±0.011 b	495.97±17.55 d	22.647±0.064 c
AzB alone H	8.87±0.75 A	0.313±0.010 C	1105.17±14.71 D	92.695±0.053 D

<sup>a</sup>)N supplied as kg ha<sup>-1</sup>

<sup>b</sup>) L – genotype low (Early golden acre)

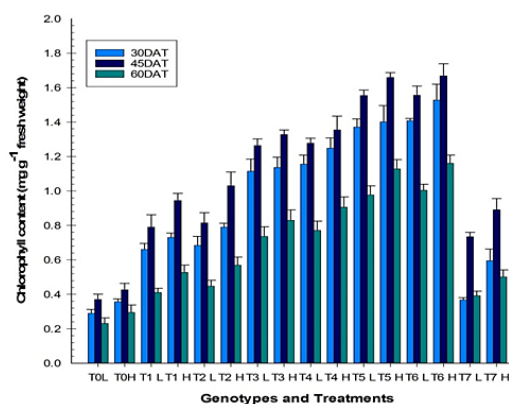
<sup>c</sup>)H – genotype high (Pusa drumhead)

<sup>d</sup>)AzB- *Azotobacter*

<sup>e</sup>)Different small case and uppercase letters denote significant difference ( $p \leq 0.05$  by DMRT) between the treatments in G-low and G-high, respectively

### Chlorophyll Content

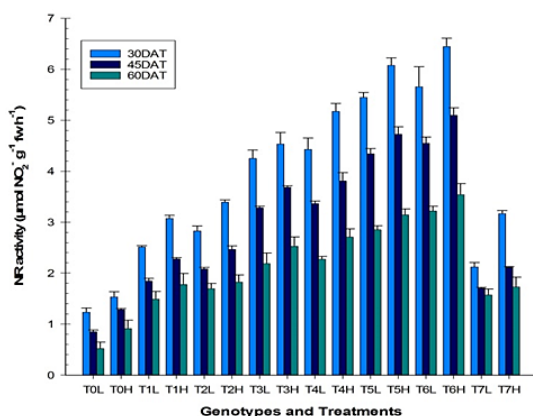
Chlorophyll content was found to increase with increasing N inputs (Fig. 2). Plants grown in N with inoculation by AzB were found to have greater chlorophyll contents than their counter parts. Genotype high (H) was found to have more chlorophyll content than genotype low (L) under all the treatments. A significant difference was observed between pre and post flowering stages of growth. At the time of pre flowering stage the chlorophyll content was found to be increased but later it was found to be decreased at flowering stages. Plants take up more nitrogen at this time so as to form many metabolites. The significant increase in chlorophyll content was due to the ability of the plant to assimilate more and more of the available N into proteins and chlorophyll molecules.



**Fig. 2: Variations in total chlorophyll content (mg g<sup>-1</sup>) in two genotypes of *Brassica oleracea* L. var. capitata grown in graded N and AzB treatments**

### Nitrate Reductase (Nr) Activity

NR activity depicted a significant ( $p \leq 0.05$ ) variation in leaves of *Brassica oleracea* grown under combined treatments of N and AzB at 30, 45 and 60 days after treatment (DAT) (Fig. 3). The leaf blade depicted the highest level of NR activity at 30 DAT followed by 45 DAT and 60 DAT. The NR activity was found to increase as we increased the application rate of N upto  $120 \text{ kg ha}^{-1}$  but further increase in N application didn't comply with increasing NR activity rather the enzyme became saturable and NR activity was found to decrease slightly in all plant parts at  $180 \text{ kg ha}^{-1}$ . It may also be due to the reason that there is an upper limit of N utilization and consequently plant performance. Moreover, there is an upper limit to the levels of N metabolizing enzymes that the plant can accommodate. Consequently, the plant continued the nitrate uptake due to its ample availability in the soil but was not able to assimilate it. This often leads to nitrate accumulation in plants.

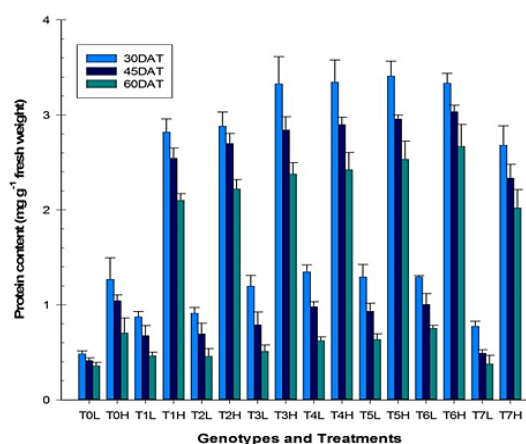


**Fig. 3:** Change in NR activity ( $\mu\text{mol NO}_2^- \text{g}^{-1} \text{f.w. h}^{-1}$ ) in leaves of two genotypes of *Brassica oleracea* L. var. capitata grown in various regimes of N and AzB at various phenological stages

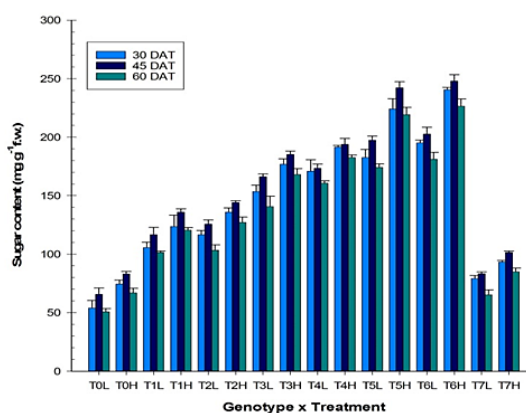
### Protein Content

The protein content increased significantly ( $p \leq 0.05$ ) as we increased the N concentration in the soil medium (Fig. 4). Plants grown under higher N regimes were found to assimilate more of the nitrate and ammonia into the proteins and hence maximal protein content was found in their leaf tissue. Protein content was found to increase significantly in plants grown in N supplemented with AzB in all the treatments. The protein content varied in the plants

at their different phenological ages. It was found to be highest at rosette stage but was found to decrease in harvest stages of plant growth.



**Fig. 4:** Changes in protein content ( $\text{mg g}^{-1} \text{f.w.}$ ) in two genotypes of *Brassica oleracea* L. var. capitata grown in graded N and AzB treatments

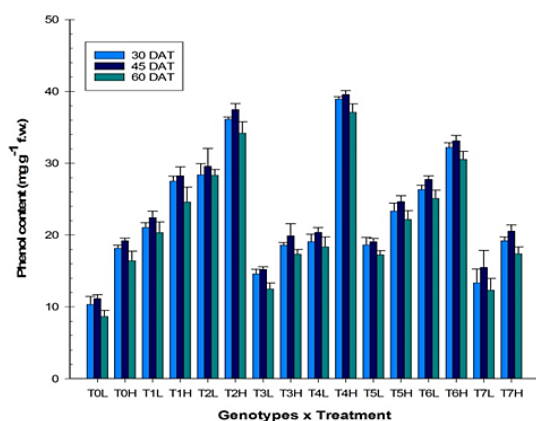


**Fig. 5:** Changes in sugar content ( $\text{mg g}^{-1} \text{f.w.}$ ) in two genotypes of *Brassica oleracea* L. var. capitata grown in graded N and AzB treatments

### Soluble Sugar Content

A significant effect of nitrogen fertilization and AzB inoculation on soluble sugar concentrations in cabbage was revealed (Fig. 5). The highest concentration of soluble sugars was found in cabbage receiving N and AzB. N fertilization applied on the rows of plants significantly increased concentration of soluble sugars in cabbage in relation to control plants. Highest sugar content was

found at  $180 \text{ kg ha}^{-1} \text{ N} + \text{AzB}$ . The increase in soluble sugars is evident from the fact that as chlorophyll increases the sugar content also increases in the plant tissue. However, the sugar content was lower in all the treatments at Harvest stages due to shift of the plant growth from vegetative to flowering period. Genotype H was found to have increasing amounts of sugar content than L in all the treatments.



**Fig. 6: Changes in phenol content ( $\text{mg g}^{-1} \text{f.w.}$ ) in two genotypes of *Brassica oleracea* L. var. capitata grown in graded N and AzB treatments**

#### Total Phenol Content

Total phenol content was found to increase with increasing N fertilizers (Fig. 6). Increase was more prominent in plants grown in combined application of N and AzB. Genotype H was found to have more phenolic content than genotype L in all the treatments. Phenolic content initially increased from initial stage to rosette stage but showed a decline at harvest stage. The treatment containing plants supplemented with AzBalone showed a significant increase in phenol content than control plants.

#### Discussion

Nitrogen is often regarded as one of the limiting factors for plant growth in agricultural systems.<sup>23</sup> Because of the key role of N in plant metabolism; one would assume plants to utilize the entire N available to them for biomass production. But, although addition of fertilizers generally results in improved yield, the effectiveness of the uptake and assimilation declines with the increasing level of fertilization.<sup>24</sup> Therefore, large amounts of fertilizers are left unused, leading to environmental and ecological

hazards (like nitrate leaching into ground water and nitrate accumulation in leafy vegetables) as well as economic loss.<sup>25</sup> Thus application of excess N fertilizers, a common agricultural practice, leads to nitrate accumulation in leafy vegetables in one hand and nitrate leaching into the ground water on other, both culminating into undesired environmental effects.<sup>26</sup>

Application of chemical fertilizer primarily targeted at enhancing its yield has not only interfered with non-targeted microbial activities, but has also affected the quality of cabbage. To reduce such deleterious effects, microbial inoculant particularly free living nitrogen fixers have been tried as an alternative or as a supplement to chemical nitrogen.<sup>27</sup> Apart from reducing the ill effects of chemical fertilizer, microbial inoculant could also maintain the quality of the cabbage and reduce the cost of cultivation. *Azotobacter*, a free living aerobic microbe has been utilized for enhancing the performance of crops by supplying them additional N requisite for their growth and productivity. Application of *Azotobacter* would reduce the dependence on inorganic sources of nitrogen. The experiment was designed to find out the effect of various levels of nitrogen and *Azotobacter* with its interaction effects to find out the best combination of different treatments to achieve maximum economic returns on growth, yield and quality of cabbage.

In this study, fresh and dry weights were found to increase significantly with an increase in N inputs. The increase was more pronounced in plants grown in integrated system of N and *Azotobacter*. Plants grown in *Azotobacter* alone were found to have fresh and dry weights significantly higher than control. The increase in fresh and dry weights may be attributed to the increasing amount of N availability which further complements plant growth. These outcomes are in accord with the opinions of Razaq *et al.*<sup>28</sup> and Kızılkaya,<sup>29</sup> who have revealed advantageous effects of bio-fertilizers on crop growth due to deliverance of growth promoting substances.

The improvement in yield characteristics as a result of using *Azotobacter* alone or in combination with N fertilizer may be due to the enhancement in vegetative growth (plant height, fresh and dry weight, leaf number, and total chlorophyll) in the treated plots. Kızılkaya<sup>28</sup> found a similar pattern in

potato plants, reporting an increase in yield due to *Azotobacter* treatment. This enhancement may be attributable to increased production of plant hormones like auxins (IAA) and gibberellins together with the vitamins (Biotin, folic acid and vitamin B groups). The processes of photosynthesis and protein synthesis were enhanced by the treatments that subsequently caused an increase in productivity per unit area. These outcomes are consistent with those of Kahlel<sup>30</sup> by growing potato crop in *Azotobacter* and N treatments.

Our results found a significant increase in NR activity with an increase in N content at rosette stage. NR activity was found to be lower in petiole followed by lower leaf and middle leaf and highest in upper leaf. Similar findings were reported by Sareer *et al.*<sup>31</sup> in *Andrographis* and Saffeullah *et al.*<sup>32</sup> in *B. oleracea*. Nitrate content also followed the reverse trend showing maximum accumulation in petiole followed by lower leaf and middle leaf and minimum accumulation in upper leaf. Our results are in agreement in Mazaharet *et al.*<sup>33</sup>

Sugar content showed an increase with increasing N treatments, highest achieved in combination with *Azotobacter*. Similar findings were reported in cabbage by Sady *et al.*<sup>34</sup>

The consumption of food having higher phenolic content can check chronic diseases associated to oxidative stress in the human body.<sup>35,36</sup> Like other phytochemicals, the level of phenolic compounds in crops, can be influenced by various factors such as cultivar used, agro-climatic features, and storage environments.<sup>34,37</sup> Our results suggest that the total phenol content increased with increasing N concentrations. Results were more pronounced in test plants treated with N + *Azotobacter*. The increase was more prominent at rosette stage and

showed a decline at harvest stage. Genotype pusa drumhead was found to contain more phenolic compounds than pusa golden acre. The level of phenolic compounds in vegetables is genotype specific though, it is greatly altered by the method and rate of N fertilization.<sup>38</sup>

### Conclusion

This study concludes that *Azotobacter* seedling treatments enhance the growth of cabbage genotypes. *Azotobacter* alone or in combination with N fertilizer significantly enhances chlorophyll content, nitrate reductase activity, protein and phenol content in cabbage plants and considerably improves the yield of the cabbage genotypes. Thus, the use of *Azotobacter* in integrated nutrient management systems is recommended which may help in the expansion of organic farming practices and will substantially lessen the cost of fertilizer application and environmental menaces caused due to larger dependency on chemical fertilizers.

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### Conflict of interest

The authors declare that they have no conflicts of interest to declare.

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