



## Quantitative assessment of broccoli (*Brassica oleracea* var *Italica*) in response to nitrogen levels

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### Abstract

Nitrogen optimization is a biggest challenge in sustainable crop production especially in fresh vegetable production. For this reason, a study was carried out for two consecutive seasons to investigate the effect of nitrogen levels on the growth, yield and nitrogen use efficiency of broccoli under the climatic conditions of Dera Ismail Khan district of Khyber Pakhtunkhwa (KPK), Pakistan. The nitrogen levels used in the study were 0, 50, 100, 150, 200, 250 and 300 kg N ha<sup>-1</sup>. The experiment was laid in randomized complete block design (RCBD) with three replicates. Parameters studied were days taken to maturity, leaf chlorophyll content, leaf number plant<sup>-1</sup>, leaf area index, head weight, head diameter, number of axillary shoots, weight of axillary shoots, total yield (t ha<sup>-1</sup>) and marketable yield (t ha<sup>-1</sup>). Results revealed a linear significant increase in growth parameters under study by addition of nitrogen at an increasing rate from 0 to 300 kg ha<sup>-1</sup> during both the years. Regression analysis of the data for all the parameters measured during both the years showed that the linear model was the best fit to the data of all parameters. The coefficient of determination for all linear models showed a strong relationship between the nitrogen rates and various parameters of broccoli. Comparing the data of two years on various parameters the t-test of significance showed that both the years were at par with each other for all the parameters studied.

**Keywords:** broccoli, nitrogen, growth, yield, correlation, coefficient of determination

### 1. Introduction

Among the macro nutrients, nitrogen has a greater impact on plant growth. It is a vital constituent of amino acids (proteins), chlorophyll, and several vitamins. Its role in enzymatic activity of plants is well understood. It improves the quantity and quality of vegetables (Silva and Uchida, 2000). On the other hand, its excessive use has a negative effect on the environment, as acid rains, global warming, eutrophication of natural systems and contamination of ground and surface water. These are all resultants of nitrogenous compounds lost from agricultural lands (Neeteson and Carton, 2001) [28]. It is reported that high demands of vegetable production in developing countries led farmers towards the excessive use of nitrogenous fertilizers to increase the yield (Moeskops *et al.*, 2010) [26]. The overuse of chemical fertilizers in Asia (Morris, 1997 [27]; Matsuda, 1998) [24], has also raised a serious concern on causing damage to the human health as well as to the environment and needs innovative contributions from crop science (Rerkasem, 2005) [34]. Di and Cameron (2002) also reported high vulnerability for nitrogen loss due to excessive use resulting in environmental degradation. Broccoli (*Brassica oleracea* L. *Italica*), a rich green vegetable, is famous for its nutritional benefits among the brassicaceae family. Being green in colour due to high chlorophyll contents, broccoli needs a large amount of nitrogen for its better growth and higher yields. According to Thompson *et al.* (2002) [31]. Nitrogen accumulation in only broccoli heads ranges from 17 to 59 % and at higher levels of 250-537 kg N ha<sup>-1</sup>, the overall accumulation of nitrogen in the total biomass of broccoli becomes 157 to 465

Kg N ha<sup>-1</sup>. Both excessive and Inadequate nitrogen application results in yield reduction or hollow stem and head rot like physiological and pathological disorders in the broccoli crop (Belec *et al.*, 2001) [9]. According to agriculture survey, the use of inorganic fertilizers is on an average 195 kg ha<sup>-1</sup> in Pakistan. The reason behind this high use is that, Pakistani soils are deficient in nitrogen due to calcareous nature and high pH level. The awareness among the agricultural product consumers of the modern world about the effect of diet on human health and the increasing frequency of major diseases like diabetes, cancer and heart problems, demands the minimum use of chemical fertilizers and improvement in the nutritional status of agricultural produce (Andow *et al.*, 2009) [4]. Therefore, for commercial and sustainable vegetable production of broccoli in Dera Ismail Khan, nitrogen optimization is highly demanded. To address this problem a two years research work was undertaken to optimize nitrogen demand of broccoli.

### Materials and Methods

#### Experimental area description

The maiden research on broccoli was conducted at the Agriculture Research Institute (ARI), Dera Ismail Khan, KPK, Pakistan, for the successive fall winter growing seasons.

#### Soil analysis

To study the pre- experiment soil status a composite soil samples from 0-30 cm depth was taken from the vegetable research area and analyzed for physico-chemical characteristics (Table.1).

**Table 1:** Soil physio-chemical properties of experimental site.

Properties	Values	
	2013	2014
Texture	Silty clay	Silty clay
pH (1:5)	8.2	8.2
Exchangeable Sodium (Na)	0.39 mmolc 100 <sup>-1</sup> g	0.40 mmolc 100 <sup>-1</sup> g
EC	0.41 (1:2.5) dSm <sup>-1</sup>	0.39 (1:2.5) dSm <sup>-1</sup>
Organic Matter	0.69 %	0.63 %
Nitrogen (N)	0.034 %	0.032 %
Phosphorus (P)	6.0 mgkg <sup>-1</sup>	7.0 mgkg <sup>-1</sup>
Potassium (K)	265 mgkg <sup>-1</sup>	278 mgkg <sup>-1</sup>

### Seed sowing

Seedlings were prepared by sowing seeds of hybrid cultivar “Rocco” in a nursery beds at 2 centimeters in shallow lines 20 cm apart.

### Land preparation, layout and transplantation

The land was prepared by ploughings and rotavated twice to break the clods. The experimental design used was randomized complete block with three repeats. Seedlings were transplanted one month after sowing on ridges at 30 and 60cm in row and between row distances, respectively. The net plot size was 3m x 4m (12 m<sup>2</sup>).

### Fertilizer application and treatments detail

A constant dose of P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O @ 80 kg ha<sup>-1</sup> was added in all plots during transplantation. To prevent N loss from leaching (Feller and Fink, 2005) [19], nitrogen was applied in two splits; first dose with 1<sup>st</sup> irrigation and remaining dose 4 weeks after transplantation as side dressing. The treatments used were T<sub>0</sub> (Control) T<sub>1</sub> (50 kg N ha<sup>-1</sup>), T<sub>2</sub> (100 kg N ha<sup>-1</sup>), T<sub>3</sub> (150 kg N ha<sup>-1</sup>) T<sub>4</sub> (200 kg N ha<sup>-1</sup>), T<sub>5</sub> (250 kg N ha<sup>-1</sup>) and T<sub>6</sub> (300 kg N ha<sup>-1</sup>).

### Cultural practices

All cultural practices recommended for cole crops according to the locality were followed uniformly including flood irrigation, single hand hoeing, earthing up etc.

### Growth and yield data

From seed sowing to apical head maturity of 50% plants, days were counted for days to maturity in each treatment. Ten randomly selected broccoli plants were taken at the time of apical head maturity from each plot to calculate the growth and yield parameters and their average was calculated. For leaf chlorophyll contents, Konica Minolta SPAD chlorophyll meter was used. Data regarding head weight and axillary shoots weight were recorded using digital balance and total yield and marketable yield were calculated using the standard formula.

### Statistical analysis

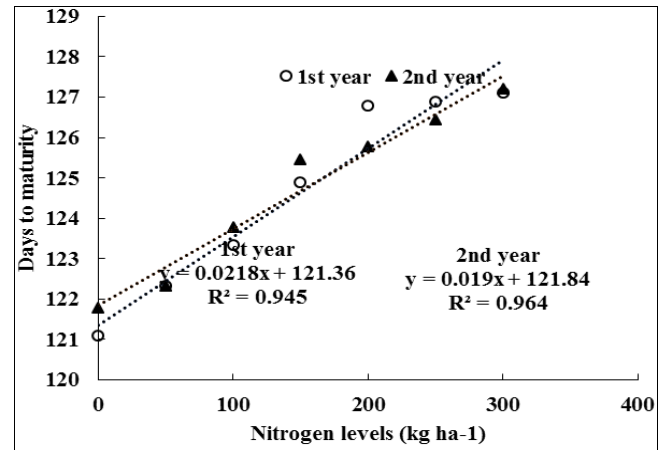
The data obtained was subjected to statistical analysis by using the analysis of variance technique given by Steel *et al.* (1997) [30]. and subsequently polynomials were fitted to the various data using computer analytical software Statistix 8.1 and the linear model, which was the best fit to various parameters, was used in this study.

## Results

### Days taken to maturity

Nitrogen levels showed significant ( $p < 0.05$ ) effect on days taken to maturity of broccoli during both the years of study.

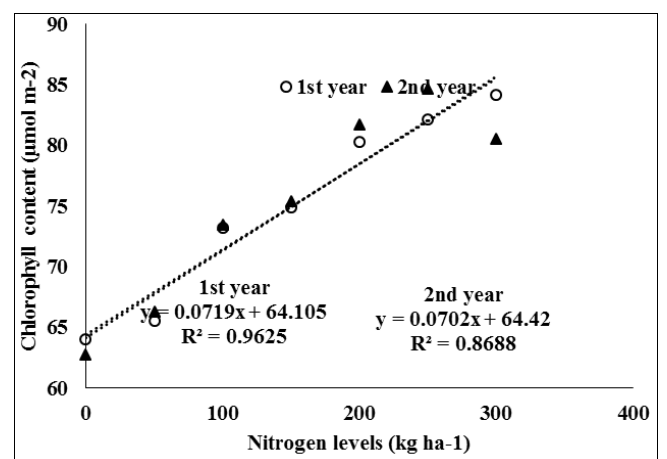
However, the co-efficient of determination for the linear model developed (Fig.1) was slightly higher during the second year ( $R^2 = 0.96$ ) than the data collected during the first year ( $R^2 = 0.94$ ). It means that every increment of nitrogen dose significantly delayed maturity during both years. Moreover, the slope of the line during both the years of study was almost similar. Broccoli plants delayed their maturity by 0.022 days per unit of nitrogen applied during both years.



**Fig 1:** Effect of increasing levels of applied nitrogen on the days to maturity of broccoli

### Leaf chlorophyll content

Chlorophyll content of broccoli leaves (Fig.2) were significantly ( $p < 0.05$ ) affected by levels of nitrogen during both the years. However, the co-efficient of determination of the linear model was higher for the data collected during the first year ( $R^2 = 0.96$ ) than the second year ( $R^2 = 0.87$ ). The chlorophyll content continued to increase with increasing nitrogen levels up to the highest nitrogen dose tested during both the years. Moreover, the slope of the line for both years was almost equal. However, the slope of the line for the first year was little bit higher than the second year. It means that for both the years, chlorophyll contents increased 0.071  $\mu\text{mol m}^{-2}$  per unit of nitrogen applied.



**Fig 2:** Effect of increasing levels of nitrogen on the chlorophyll content ( $\mu\text{mol m}^{-2}$ ) of broccoli

### Numbers of leaves (plant-1)

It is a well-established fact that increasing the nitrogen levels in plant promotes vegetative growth. Results showed significant ( $p < 0.05$ ) increase in broccoli leaves by the

increment of nitrogen levels during both years. Regression equations developed from the data of two years (Fig.3) shows that the co-efficient of determination for the linear model during the first year was  $R^2 = 0.96$  and during the second year, it was  $R^2 = 0.95$ . The equation shows that leaf number increased by the addition of nitrogen doses reaching

their maximum at highest dose applied during both the years. Moreover, the slope of the line for both the years was equal. It means that number of leaves increased by 0.057 per unit of nitrogen applied for the first year, while it was 0.059 for the second year.

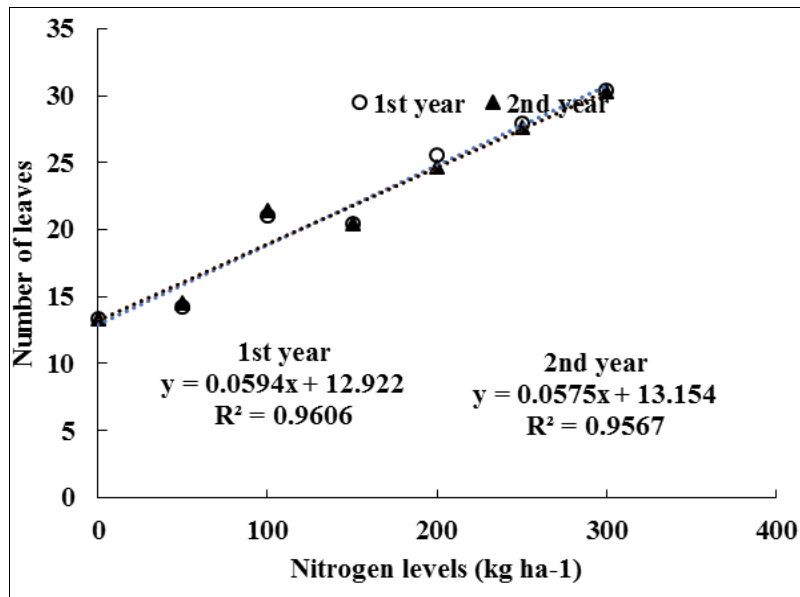


Fig 3: Effect of various levels of nitrogen on the number of leaves plant<sup>-1</sup> of broccoli

**Leaf area index**

Data collected on the leaf area index of broccoli showed significant ( $p < 0.05$ ) effect of nitrogen levels during both the years. The co-efficient of determination for the linear model (Fig. 4) was almost similar ( $R^2 = 0.90$ ) for the data collected during both the years. Strong trend of the regression equation shows a linear increase in LAI by every increment of nitrogen applied up to the highest amount. Moreover, the slope of the line for both the years was almost similar. It means that LAI increased 0.004 per unit of nitrogen applied.

**Head Diameter of broccoli during both years.**

The co-efficient of determination for the linear model (Fig. 5) for the data collected during the first year was higher ( $R^2 = 0.81$ ), than the co-efficient of determination for the second year ( $R^2 = 0.70$ ). Regression equation also indicates that nitrogen level of 200 kg ha<sup>-1</sup> significantly produced larger heads in broccoli. Moreover, the slope of the line for the first year was 0.014 as against 0.016 for the second year. It means that head diameter increased 0.014 to 0.016 cm per unit of nitrogen applied.

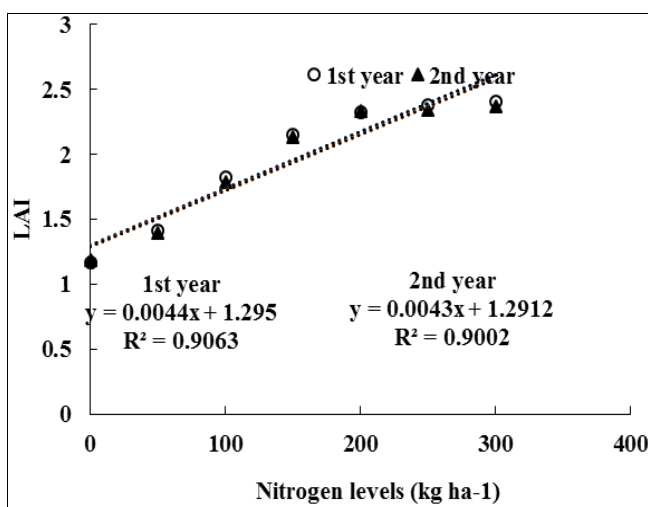


Fig 4: Effect of increasing levels of applied nitrogen on the leaf area index (LAI) of broccoli

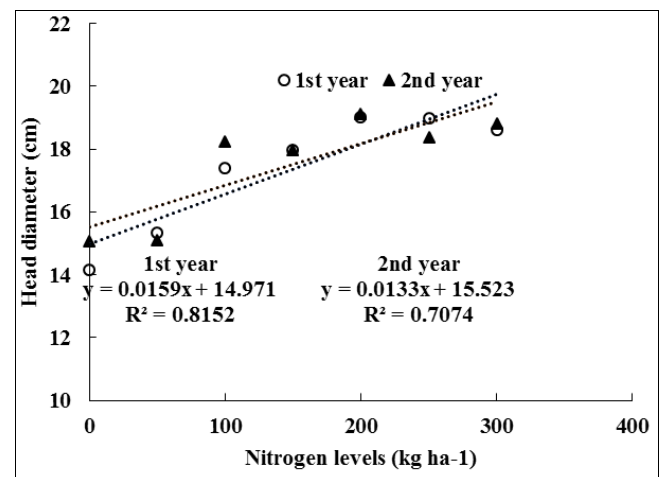


Fig 5: Effect of increasing levels of applied nitrogen on the head diameter of broccoli

**Head diameter (cm)**

In broccoli, larger heads are preferred over the smaller one. Application of nitrogen significantly ( $p < 0.05$ ) increased

**Head weight (g)**

Significant differences were recorded during both the years regarding head weight of broccoli under different nitrogen levels. The co-efficient of determination for the linear model (Fig.6) developed for the data of two years was slightly

Higher for the first year ( $R^2 = 0.90$ ) than the co-efficient of determination ( $R^2 = 0.82$ ) for the second year. It means that the relationship between head weights and increasing levels of N was strong during both the years. Head weight of broccoli continued to increase up to 200 kg ha<sup>-1</sup> nitrogen and then showed a stable trend or slight decline by

increasing the nitrogen up to the highest level. Moreover, slope of the line for both the years was almost similar. However, the slope of the line for the first year was little bit higher (1.173 g per unit of N applied) than the second year (1.140 g). It means that weight of head increased per unit of nitrogen applied linearly during both the years.

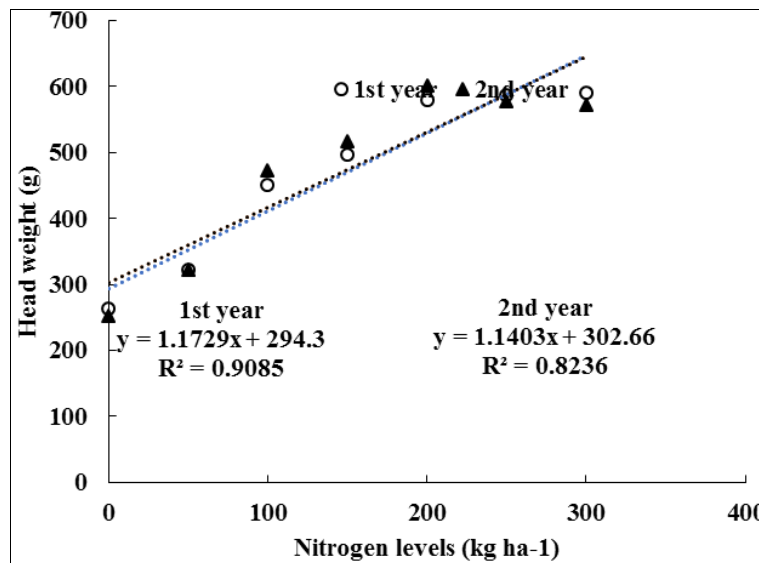


Fig 6: Effect of increasing levels of applied nitrogen on the head weight of broccoli

**Number of axillary shoots**

In broccoli after the harvest of apical heads, axillary shoots/lateral shoots/lateral heads/axillary heads or side shoots start to grow and increase in size from the axil of leaves. Number of axillary shoots significantly ( $p < 0.05$ ) increased with the application of nitrogen during both years. The co-efficient of determination for the linear model (Fig. 7) developed for the two years data was  $R^2 = 0.96$  and  $R^2 = 0.93$  during the first year and the second year of study, respectively. It means that axillary shoots showed strong linear trend in terms of increase in number by the application of increasing nitrogen rates. Moreover, the slope of the line for both the years was almost similar, showing increase in number of axillary shoots 0.011 per unit of nitrogen applied during the first year as against 0.10 per unit of nitrogen applied during the second year.

**Weight of axillary shoots (g)**

Weight of axillary shoots was also increased by the addition of nitrogen. The co-efficient of determination for the linear model (Fig. 8) was  $R^2 = 0.96$  and  $R^2 = 0.94$  for the data collected during the first and second years, respectively. Strong trend of the regression equation shows a linear increase in axillary shoots weight by the increment of nitrogen application up to the maximum dose applied. Moreover, the slope of the line for both the years was almost similar. It means that weight of axillary shoots increased 1.66 g per unit of nitrogen applied during the first year and 1.69 g per unit of N applied during the second year.

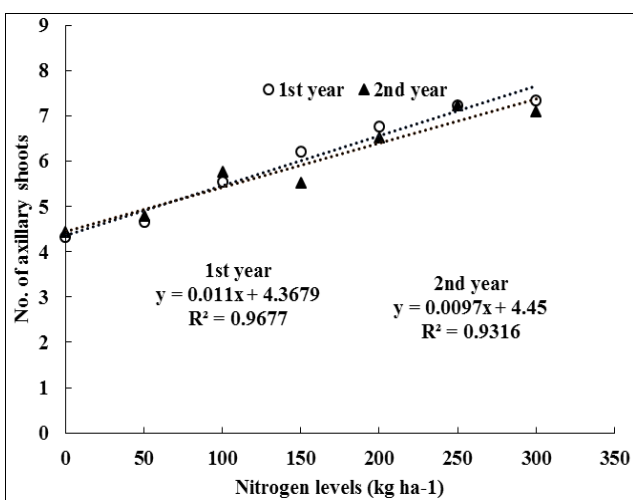


Fig 7: Effect of increasing levels of nitrogen on the number of axillary shoots of broccoli

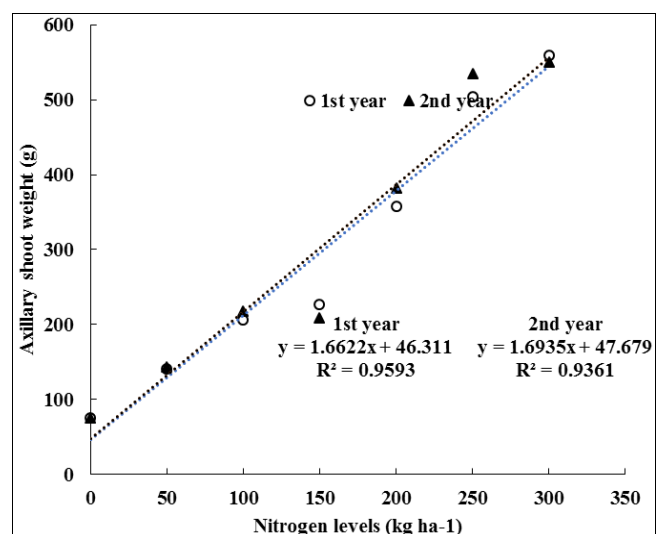
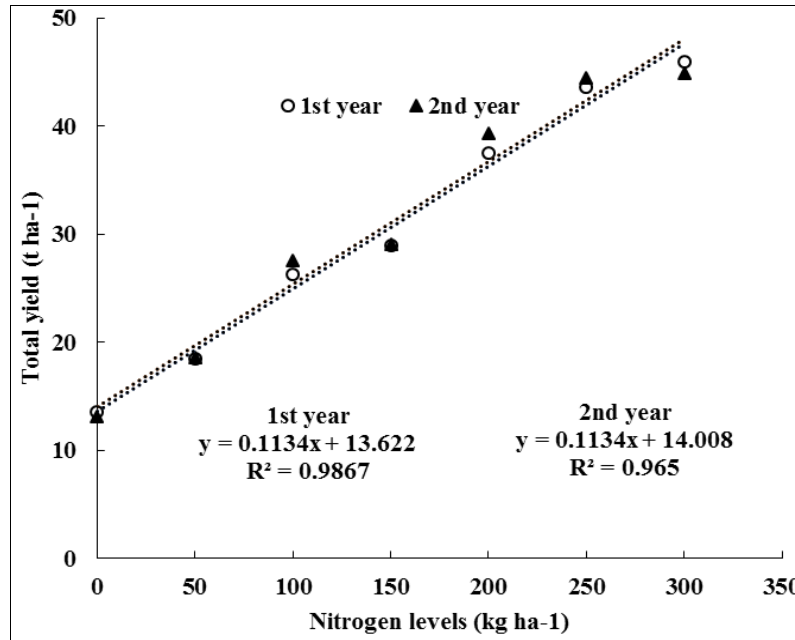


Fig 8: Effect of increasing levels of nitrogen on the weight of axillary shoots of broccoli

**Total yield (t ha<sup>-1</sup>)**

Total yield is the outcome of total cultivable area of land by the crop. Data showed significant ( $p < 0.05$ ) effect of treatments on the total yield (tons ha<sup>-1</sup>) of broccoli during both the years of study. However, the co-efficient of determination for the linear model (Fig.9) during the first year was slightly higher ( $R^2 = 0.98$ ) than the co-efficient of

determination ( $R^2 = 0.96$ ) for the second year. Model shows a linear increase in attaining total yield (tons ha<sup>-1</sup>) with an increase of nitrogen level. Moreover, the slope of the line showed same trend during both the years. It means that total yield increased by 0.11 tons ha<sup>-1</sup> per unit of nitrogen applied.



**Fig 9:** Effect of increasing rates of nitrogen on the total yield (t ha<sup>-1</sup>) of broccoli

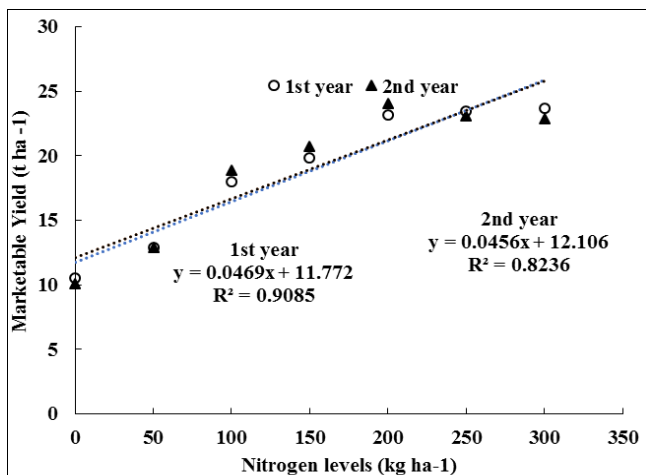
**Marketable yield (t ha<sup>-1</sup>)**

Marketable yield of broccoli depends on the size and weight of apical heads. The coefficient of determination of the linear model (Fig.10) developed for the data collected on the marketable yield of broccoli during the first year was slightly higher ( $R^2 = 0.90$ ) than the coefficient of determination ( $R^2 = 0.82$ ) of the second-year. Regression equation also showed a linear increase in marketable yield up to 200 kg N ha<sup>-1</sup> and then a slight decline was recorded for upper nitrogen levels. Moreover, the slope of the line for the data collected during both the years of study was almost equal (0.046). It means that marketable yield increased by 0.046 tons ha<sup>-1</sup> per unit of nitrogen applied for both the years.

The increase in various yield parameters can be explained based on strong relationship between the growth parameters and the yield parameters (Table 2.). It can be visualized from the r-values between the growth parameters and the yield parameters that there was a strong relationship between these two parameters. As the various growth parameter increases, there was a corresponding increase in various yield parameters.

**Table 2:** Correlation (r-values) between various yields (t ha<sup>-1</sup>) and different growth parameters

Growth Parameters	Total yield	Marketable yield
Days to maturity	0.98	0.97
Leaf chlorophyll	0.99	0.99
Number of axillary shoots	1.00	0.96
Number of leaves	0.99	0.94
Head diameter	0.91	0.98
Head weight	0.95	1



**Fig 10:** Effect of increasing rates of nitrogen on the marketable yield (t ha<sup>-1</sup>) of broccoli

Stepwise regression analysis of the data was performed keeping marketable yield as dependent variable, and the other growth factors as independent variables. The following model was developed:

$$\text{Marketable yield (t ha}^{-1}\text{)} = -271.039 + 2.33(\text{Days taken to maturity}) \quad R^2 = 0.94$$

It can be visualized from this expression that the major growth factor contributing towards marketable yield is days taken to maturity.

**Discussion**

Nitrogen promotes vegetative growth in plants hence delaying the maturity in plants (Jana and Mukhopadhyay,

2001) [21]. It might be due to availability of sufficient assimilates triggering high photosynthetic activity, thereby switching the plant towards vegetative growth instead of reproductive phase. Extension of vegetative growth period due to excessive N supply and delay in maturity was also reported earlier (Mengel and Kirkby, 1987) [25]. According to Lamb *et al.* (2012) [23], in plant experimental research, measurement of chlorophyll content is an important parameter. As nitrogen is an important segment of chlorophyll molecule (Daughtry *et al.*, 2000 [14]; Tucker, 2004) [36], it is logical that increasing levels of N will increase the chlorophyll content of broccoli. The same trend has been observed by other research workers who found that increase in chlorophyll content of leaves mostly depends on the supply of nitrogen and other plant nutrients. Present findings are well endorsed with the results of Zhang *et al.* (2017) [38], who recorded high chlorophyll contents by increasing N and P levels in cabbage seedlings. In vegetable crops, trend of increase in leaf number by increasing the rate of nitrogen has been well documented (Biemond *et al.*, 1995) [8]. Abou El-Magd *et al.* (2015) [1], and Dhakal *et al.* (2016) [15], also reported highest number of leaves in broccoli and Chinese cabbage with the highest level of nitrogen applied. It was due to the synthesis of more cytokinin in the root system of plant under optimum nitrogen availability, which in turn triggers the leaf growth (Van der Werf and Nagel, 1996). The ascending trends in the leaf area of broccoli was also reported by Dhakal *et al.* (2016) [15], with 0 to 240 kg N ha<sup>-1</sup>, which strongly supports the presented results. Earlier, Vagen *et al.* (2004) reported lower LAI accompanied by lower yields by decreasing the N levels. As far as the head size is concerned, large and compact heads are preferred over the undeveloped and loose ones. Brahma *et al.* (2002) [13], Reported maximum head diameter of 19.52 cm in broccoli at 200 kg N ha<sup>-1</sup>. Apical heads are the main marketable commodity produced in broccoli. Greater the head weight more the marketable yield and hence greater return to the farmers. Nitrogen has a strong effect on the head weights of brassicaceae family. Maximum head weight in broccoli was recorded by Agarwal *et al.* (2007) [3], at nitrogen level of 200 kg ha<sup>-1</sup>. Easmin *et al.* (2009) obtained maximum head weight of Chinese cabbage at 250 kg N ha<sup>-1</sup> and minimum at control. Bjelic *et al.* (2005) and Bozkurt *et al.* (2011) [12]. Reported increasing trend in curd weight of cauliflower with increasing levels of nitrogen from 0 to 225 kg ha<sup>-1</sup>. The slight difference in head weight though non-significant during the two years might be due to different temperature regimes as reported by Feller and Fink (2005) [19], as broccoli is more thermosensitive than other vegetables. Drost and Johnson (2005) [16]. Reported that development of side shoots is dependent on the appropriate supply of nitrogen. Dhakal *et al.* (2016) [15]. Recorded maximum number of axillary shoots at the level of 240 kg N ha<sup>-1</sup> while minimum was recorded in control plots. Similar results were reported by Dhakal *et al.* (2016) [15], in their study with broccoli that increase in the nitrogen levels significantly increased axillary shoots weight. A significant effect of increasing the nitrogen levels on the total yield of broccoli up to the application of 240 and 250 kg N ha<sup>-1</sup> were also reported by Dhakal *et al.* (2016) [15], and Kowalenko and

Hall (1987) [22], respectively. Whereas, a highest total yield of 18.11 t ha<sup>-1</sup> was reported by Brahma *et al.* (2002) [13], at 200 kg N ha<sup>-1</sup>. Marketable yield is the profitable outcome of total inputs provided to a crop during the entire course of growing period. In broccoli, apical heads are the main marketable products due to their attractive green compact appearance, while the axillary shoots are not usually marketed. According to Everaarts and De Willigen (1999) [18], nitrogen application is essential to obtain maximum yield of marketable heads. Zearth *et al.* (1995) [37] and Thompson *et al.* (2002) [31]. Reported maximum marketable yield with the application of 435 to 560 kg N ha<sup>-1</sup> and 300 to 500kg N ha<sup>-1</sup>, respectively. Bakker *et al.* (2009) [7]. Recommended 298 to 309 kg N ha<sup>-1</sup> for economical production of broccoli but at rates of 200 kg N ha<sup>-1</sup> plants showed maximum recovery of available nitrogen and minimum loss to the soil. Dhakal *et al.* (2016) [15]. Concluded that at higher rates of nitrogen application, plant overall biomass increased but there was no significant increase in marketable yield of broccoli. Nitrogen is responsible for extending the vegetative growth period and delaying the maturity of broccoli and other crops (Jana and Mukhopadhyay, 2001) [21]. The longer it takes to maturity, more photo synthates will be manufactured in the leaves and transported to the other parts resulting in more yields (Gautam *et al.*, 1998) [20].

### Conclusions and recommendations

The present research work concludes that increasing levels of nitrogen significantly and linearly increased various growth and yield parameters of broccoli and linear model was the best fit to the data of all parameters during both the years of study. Relationship between the nitrogen rates and various parameters of broccoli studied was very strong with very high coefficient of determination. Stepwise regression of the data showed that days taken to maturity of broccoli was the major vegetative growth factor contributing towards marketable yield of broccoli. For marketable yield 200 kg N ha<sup>-1</sup> is recommended, however, rate of nitrogen can be adjusted using yield models depending upon the yield targets.

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### Authors Contributions

Asif Latif: Carried out research work  
 Muhammad Saleem Jilani: Supervised the research  
 Mohammad Safdar Balooch: Worked as co-supervisor  
 Malik Muhammad Hashim: Helped in analysis and writeup

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