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Influence of differential nitrogen application on biochemical, physiological parameters and tuber yield in two potato cultivars

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Abstract

Potato (*Solanum tuberosum* L.) is a globally significant food crop, valued for its high energy content and essential nutrients. This study investigates the effects of nitrogen fertilization on vegetative growth and biochemical composition in potato plants. Key vegetative traits assessed include plant height, shoot number, shoot biomass, and nitrogen content, while biochemical parameters include protein, starch, reducing sugars, total sugars, specific gravity, dry matter, and vitamin C. Results showed that nitrogen fertilization significantly enhanced vegetative growth, increasing plant height, shoot number, and biomass, along with higher nitrogen content in plant tissues. Biochemically, nitrogen application led to increased protein content, reducing sugars, and total sugars. However, excessive nitrogen negatively affected tuber quality, decreasing starch content, dry matter, specific gravity, and vitamin C levels. These findings suggest that while nitrogen supports vigorous growth and enhances some nutritional components, over-application can compromise tuber quality. Therefore, balanced nitrogen management is essential for maximizing both yield and nutritional value in potato production. Statistical analysis was conducted using SPSS, while graphical data representation was carried out using Python and Minitab 18 (Minitab Inc., State College, PA, USA). This study underscores the importance of optimizing nitrogen input to achieve sustainable and high-quality potato cultivation.

Keywords: Nitrogen management; biochemical factors; vegetative growth; nutritional value

Introduction

Potato (*Solanum tuberosum*) is a widely cultivated crop with significant nutritional value and adaptability to diverse agro-ecological environments (Ahmadu *et al.*, 2021) [3]. Native to the Andes, it has become a global food staple (Liu *et al.*, 2021) [32]. Potatoes are mainly composed of starch, which constitutes the majority of their carbohydrate content, serving as a major energy source (Sawicka and

Gupta, 2018). In addition to starch, potatoes provide essential proteins, approximately 2% by weight, including amino acids like lysine (Ralet and Gueguen, 1999) [49]. While not considered a high-protein food, potatoes are an important source of plant-based protein, critical for growth and metabolic processes (Bhutto *et al.*, 2024) [10]. They also contain reducing sugars, such as glucose and fructose, which result from starch hydrolysis, further contributing to their nutritional profile (Singh *et al.*, 2023) [60]. Together, the starch, protein, and sugars in potatoes create a balanced food source providing both energy and essential nutrients.

The nutritional quality and yield of potatoes are influenced by several environmental and agronomic factors, with nitrogen being one of the most crucial macronutrients (Muleta and Aga, 2019) [38]. Nitrogen plays a key role in the biosynthesis of proteins, starch, and sugars, all of which are integral to the potato's nutritional composition. As an essential nutrient, nitrogen is incorporated into amino acids, such as lysine, which are vital for protein synthesis and other physiological functions (Shah *et al.*, 2024; Naseer *et al.*, 2019) [58, 40]. Nitrogen availability also regulates starch biosynthesis, affecting the potato's energy content and physical characteristics (Shaikh *et al.*, 2024) [59]. Additionally, nitrogen is involved in the formation of reducing sugars during starch breakdown. Therefore, optimal nitrogen management is necessary to enhance both the nutritional quality and yield of potatoes, ensuring a productive and nutrient-dense crop (Maheswari *et al.*, 2017) [34].

Nitrogen is fundamental to the biosynthesis of proteins, starch, and sugars in potatoes, directly impacting their growth, yield, and biochemical composition. As an essential macronutrient, nitrogen is a key component of amino acids, the building blocks of proteins (Leghari *et al.*, 2016) [29]. Adequate nitrogen availability promotes the synthesis of proteins, including lysine, which is often a limiting factor in plant-based diets (Kandi *et al.*, 2012) [25]. These proteins are critical for various physiological processes, including cellular metabolism, tissue growth, and immune function (Sudhakararao *et al.*, 2019) [64]. Nitrogen also influences starch formation by regulating the enzymes involved in starch biosynthesis (Valiullin *et al.*, 2024) [68]. Higher nitrogen levels typically promote increased starch accumulation, improving the potato's energy density and texture. However, excessive nitrogen can reduce starch content (Li *et al.*, 2018) [31]. Nitrogen also contributes to the production of reducing sugars during starch degradation, further enhancing the potato's nutritional quality (Roe *et al.*, 1990) [52]. Thus, effective nitrogen management is essential for optimizing both yield and biochemical quality in potatoes. The nitrogen content in potatoes is vital not only for biochemical processes but also for shoot biomass accumulation (Vos, 2009; Qi and Pan, 2022) [70, 48]. Sufficient nitrogen encourages robust shoot growth, which is crucial for plant development, tuber formation (Bélanger *et al.*, 2001) [8] and the synthesis of key compounds such as proteins, starch, and sugars (Blumenthal *et al.*, 2008) [11]. Nitrogen supports protein synthesis, including essential amino acids like lysine (Causin, 1996) [12] and regulates starch biosynthesis, thereby enhancing the energy content of the tuber. On the physiological level, nitrogen facilitates optimal shoot biomass accumulation, improving plant health and overall yield (Kubar *et al.*, 2021; The *et al.*, 2021) [27, 67]. In contrast, insufficient nitrogen reduces shoot biomass, hinders plant growth, and leads to lower protein and starch content. Effective nitrogen management is key to maximizing both shoot biomass (Ren *et al.*, 2023) [51] and the biochemical quality of potatoes, ensuring improved yield and nutritional value (Darabi *et al.*, 2018) [14]. Therefore, this study investigates how two potato cultivars respond to varying nitrogen application rates, aiming to optimize

nitrogen use for better crop performance, nutritional quality, and more efficient agricultural practices, ultimately contributing to global food security.

2. Materials and Methods

2.1 Experimental site and location

The field experiment was conducted in October 2023 at two locations: the Department of Soil Science at Punjab Agricultural University (PAU) (30.91°N, 75.85°E) and the University Seed Farm in Naraingarh (30.35°N, 76.15°E). Both sites have well-drained sandy loam soils, with mean annual rainfall of 733 mm and 757 mm, respectively, and experience a subtropical climate with temperatures ranging from 17 °C to 32 °C, favourable for potato cultivation. A split-plot design with three replications was used, with varying nitrogen levels (T1-N0=control, T2-N1=62.5 kg/ha, T3-N2=125 kg/ha, T4- N3=187.5 kg/ha, T5 – N4=250 kg/ha, T6-N5=312.5 kg/ha, T7-N6=375kg / ha) assigned to the main plots and two potato varieties, Punjab Potato 102 and Kufri Pukhraj, kept in subplots. Nitrogen was applied as neem-coated urea in two equal splits, at 3 and 6 weeks after planting, with diammonium phosphate (DAP) applied initially, except for the control, which received single super phosphate. Phosphorus (62.5 kg/ha) and potassium (62.5 kg/ha), along with farmyard manure (50 t/ha), were added as per PAU guidelines.

2.2 Soil analysis

Soil samples were collected 10 days before crop sowing and field operations began. These samples were analyzed for physical properties, including sand, silt, and clay percentages, as well as chemical parameters such as pH, electrical conductivity (EC), organic carbon (OC), and concentrations of nitrogen (N), phosphorus (P), potassium (K), and micronutrients (Fe, Mn, Cu, Zn). Particle size distribution was determined using the method of Piper's method, while soil nutrient levels were assessed using standard protocols: nitrogen by Subbiah and Asija's method, phosphorus by Olsen's method, potassium by Jackson's method and micronutrients (Fe, Cu, Mn, Zn) by Lindsey and Norwell's method, described by Rayment and Lyons, (2011) [50], shown in table 1.

Table 1: Soil analysis of experimental sites (0-15 cm) at ludhiana and naraingarh

Component	Ludhiana	Naraingarh
Physical properties		
Sand%	74.1	76.3
Silt%	16.6	14.8
Clay%	9.3	8.9
Soil texture	Sandy loam	Sandy loam
Chemical properties		
Electrical conductivity (dSm ⁻¹)(1:2)	0.16	0.14
pH (1:2)	7.12	7.48
Organic Carbon (%)	0.34	0.31
Available N (kg ha ⁻¹)	69.0	97.4
Available P (kg ha ⁻¹)	15.3	17.3
Available K (kg ha ⁻¹)	94.5	104.2
Available Fe (kg ha ⁻¹)	52.6	47.7
Available Mn (kg ha ⁻¹)	36.8	39.5
Available Zn (kg ha ⁻¹)	9.6	6.4
Available Cu (kg ha ⁻¹)	4.5	5.7

2.3 Performance Indicators

2.3.1 Biochemical parameters

Proteins

Protein estimation in potato was performed using the Trichloroacetic Acid (TCA) method, used by Niamke *et al.*,

(2005) [41]. A 1 g dry potato sample was homogenized in 10 mL of 0.1 M phosphate buffer (pH 7.0) and centrifuged at 12, 000×g for 15 minutes at 4 °C. To precipitate proteins, 500 µL of the supernatant was mixed with 500 µL of 10% TCA and incubated on ice for 30 minutes, followed by

centrifugation at 12,000×g for 10 minutes. The protein pellet was washed twice with 5% TCA and resuspended in 0.1 M NaOH. Protein concentration was determined by reacting 100 µL of the sample with Biuret reagent, and absorbance was measured at 280 nm. Protein content was calculated using a standard curve prepared with bovine serum albumin (BSA).

Starch, Reducing sugars and Total sugars

Starch content was estimated using the McCready method, previously used by Thakur *et al.*, (2022) ^[66] where 0.5 g of ground potato was mixed with 20 mL of 80% ethanol to remove soluble sugars. The residue was treated with distilled water and heated in a boiling water bath to gelatinize the starch. Starch hydrolysis was achieved by adding 9 N HCl and incubating at 100 °C for 2 hours, followed by neutralization with 1 N NaOH. The final volume was adjusted, and iodine solution was added for colour development. Absorbance was measured at 620 nm, and starch concentration was determined using a standard glucose curve.

Reducing sugars were estimated using the dinitro salicylic acid (DNS) method, previously used by Dusabe *et al.*, (2023) ^[15]. A 1 g potato sample was homogenized in 10 mL distilled water, centrifuged, and filtered. To 1 mL filtrate, DNS reagent was added, and the mixture was heated for 5 minutes. After cooling, potassium sodium tartrate was added to stabilize the colour. Absorbance was measured at 540 nm, and reducing sugar concentration was calculated using a glucose standard curve.

Total sugars were determined using the Dubois method, previously used by Chen *et al.*, (2023) ^[13] where 1 g of potato was homogenized, centrifuged, and filtered. To 1 mL of the extract, 1 mL of 5% phenol and 5 mL sulfuric acid were added, forming a yellow-orange colour. Absorbance was measured at 490 nm, and total sugar concentration was determined using a glucose standard curve.

Crude protein

Crude protein content was determined using the Kjeldahl method, described by Kalra, (1998) ^[24]. A 1 g potato sample was digested with 20 mL of concentrated sulfuric acid (H₂SO₄) and a catalyst (selenium or copper) at high temperatures (around 400 °C) until the solution became clear. The digested sample was then cooled, diluted with water, and treated with 40% sodium hydroxide (NaOH) for distillation. Ammonia released during distillation was absorbed in 4% boric acid solution, and ammonia content was quantified by titration with 0.1 N hydrochloric acid (HCl). Nitrogen content was calculated from the titrant volume, and crude protein content was determined by multiplying nitrogen by a factor of 6.25.

Dry matter

Dry matter content was determined by weighing a fresh potato sample, washing, peeling, and reweighing it. The tubers were dried in an oven at 65 °C for 48 hours or until a constant weight was achieved. The dry weight was measured, and dry matter content was calculated as the percentage of dry weight relative to the fresh weight.

Specific gravity

Specific gravity was determined using the water displacement method. The fresh weight of the tubers was

measured, and they were submerged in water. The volume displaced by the tubers was recorded, and specific gravity was calculated as the ratio of the fresh weight to the displaced water volume.

2.3.1.6 Vitamin C content

Vitamin C content was determined using the method described by Nielsen, (2024) ^[42]. Potato tubers were homogenized and extracted with metaphosphoric acid to prevent oxidation. The extract was filtered and diluted, and an aliquot was titrated with 2, 6-dichlorophenol-indophenol solution. The endpoint, indicated by a colour change from blue to colourless, marked the reduction of the indophenol dye by ascorbic acid. Vitamin C content was calculated using a standard curve of known ascorbic acid concentrations, expressed as milligrams per 100 g of fresh weight.

2.3.2 Physiological parameters

Plant height and number of shoots

Plant height and shoot number were measured at 40 days after sowing (DAS) and at harvest. Five plants per plot were randomly selected at each time point, and plant height was measured from the soil surface to the apex of the tallest stem or leaf using a graduated ruler. The average height per plot was calculated. Shoot number was determined by counting all shoots, including lateral branches, on five plants per plot. These measurements were taken in the morning to minimize environmental influence.

Shoot biomass and N content

Biomass and nitrogen (N) content were assessed at 7-day intervals from 28 to 63 DAS. Shoots were collected, washed, and dried at 70 °C for 48 hours to determine dry weight, recorded as grams per plant. Nitrogen content was analyzed by digesting 0.5 g of dried biomass with sulfuric acid and catalysts, followed by neutralization and distillation into hydrochloric acid. The distillate was titrated with NaOH, and nitrogen content was calculated from the volume of titrant used, expressed as milligrams of nitrogen per gram of dry biomass.

Tuber yield

Tuber yield was determined by harvesting potatoes from a pre-determined plot area, typically measured in hectares. At harvest, plants were carefully uprooted, and the tubers were separated from the soil and cleaned to remove any adhering dirt. The total fresh weight of the tubers was recorded as the tuber yield per unit area, expressed in tons per hectare.

3. Results

Proteins are essential for plant stress responses, energy storage, nutrient utilization, and pathogen resistance, which are crucial for growth, survival, and adaptability (Kapoor *et al.*, 2015) ^[26]. The application of N significantly increased protein content in potatoes. In PP102, the average protein content was 1.50% at L1 (Ludhiana) and 1.62% at L2 (Naraingarh), while in Kufri Pukhraj, it was 1.16% and 1.14%, as shown in Table 2. The highest protein content in PP102 was observed in T7 (2.20% and 2.24%), which was statistically comparable to T5 (1.92% and 2.17%). For Kufri Pukhraj, the maximum protein content was recorded in T7 (1.65% and 1.69%), which did not differ significantly from T4.

Starch, reducing sugars, and total sugars are crucial carbohydrate constituents in potatoes, influencing plant metabolism and food production (Sowokinos, 2007) [62]. The effect of nitrogen on both varieties was significant, with PP102 averaging starch content at 11.9% and 11.7%, while Kufri Pukhraj averaged 10.6% at both locations (Table 2). The maximum starch content in PP102 was recorded in T1 (14.1% and 14.0%) at L1 (Ludhiana) and L2 (Naraingarh), comparable to T4 (12.3%) and T2 (13.4%). For Kufri Pukhraj, T1 showed the highest starch content (11.7% and 11.8%), similar to T5 (10.7%) and T4 (11.1%). Reducing sugars, including glucose, fructose, and maltose, play a key role in starch hydrolysis (Singh *et al.*, 2010) [61] and affect the flavour, texture, and colour of potato-based food products (Miranda and Aguilera, 2006) [37]. The average reducing sugars content was significantly higher in Kufri Pukhraj, averaging 649.3 mg/100g and 647.2 mg/100g, compared to PP102, which averaged 147.4 mg/100g and 146.9 mg/100g (Table 2). In PP102, the highest reducing sugars content was recorded in T7 (157.8 mg/100g and 155.9 mg/100g), comparable to T4 (149.2 mg/100g) and T5 (151.5 mg/100g), while for Kufri Pukhraj, T7 showed the highest content (698.8 mg/100g and 695.3 mg/100g), significantly higher than all other treatments. Total sugars, comprising monosaccharides and disaccharides, are essential for the plant's metabolic functions (Figueroa *et al.*,

2021) [18] and influence the quality of processed products (Pokrzywnicka and Koncki, 2018) [47]. The nitrogen effect was significant on total sugars, with Kufri Pukhraj averaging 3.16 g/100g and 3.17 g/100g, and PP102 averaging 1.63 g / 100g and 1.51 g / 100g (Table 2). The highest total sugar content in PP102 was recorded in T7 (2.07 g / 100g and 1.94 g / 100g), statistically comparable to T4 (1.55 g / 100g) and T6 (1.72 g/100g). In Kufri Pukhraj, T7 showed the highest total sugar content (3.75 g / 100g and 3.58 g/100g), comparable to T5 (3.37 g/100g) and T6 (3.40 g/100g). *Dry matter* content in potatoes is a crucial factor influencing quality, nutritional value, and processing characteristics (Naeem and Caliskan, 2020) [39]. It impacts properties such as texture, flavour, and cooking behaviour, making it essential for determining suitability for various culinary and industrial applications. Additionally, dry matter affects the nutritional profile by influencing carbohydrate levels and other key metabolites. Optimizing dry matter content is vital for improving yield and food quality in potato cultivation (Islam *et al.*, 2022) [23]. For the PP102 variety, the highest dry matter content was recorded in T1 (17.7 g/100g and 17.9: g / 100g), comparable to T4 (16.9 g/100g) and T3 (17.1 g/100g) at both L1 and L2. In Kufri Pukhraj, the maximum dry matter was observed in T1 (14.7 g/100g and 14.5 g / 100g), similar to T2 (13.9 g/100g) and T3 (13.6 g/100g).

Table 2: Biochemical parameters and tuber yield as affected by different nitrogen treatments at ludhiana and naraingarh.

	Treatment s	Proteins (%)		Starch (%)		Reducing Sugars (mg/100g)		Total Sugars (g/100g)		Dry matter (%)		Specific Gravity		Crude Protein (%)		Vitamin C (mg/100g)		Tuber Yield (t ha ⁻¹)	
		V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2
Ludhiana	T1	0.45 ^a	0.29 ^a	14.1 ^a	11.7 ^a	136.4 ^a	596.3 ^a	1.09 ^a	2.59 ^a	17.7 ^a	14.7 ^a	1.08 ^a	1.07 ^a	5.8 ^a	5.0 ^a	17.9 ^a	42.6 ^a	17.0 ^a	15.0 ^a
	T2	0.95 ^b	0.70 ^a _b	13.5 ^a _b	11.4 ^a _b	138.8 ^a	614.6 ^b	1.38 ^{ab}	2.84 ^{ab}	17.1 ^{ab}	13.9 ^a _b	1.08 ^a	1.06 ^{ab}	10.8 ^b	7.4 ^{ab}	15.9 ^{ab}	38.4 ^b	22.3 ^b	19.9 ^b
	T3	1.29 ^c	1.00 ^{bc}	12.8 ^a _b	11.1 ^a _b	143.7 ^a _b	634.4 ^c	1.45 ^{abc}	3.00 ^{abc}	17.0 ^{ab}	13.2 ^b	1.07 ^{ab}	1.06 ^{abc}	11.9 ^{bc}	8.5 ^{bc}	14.7 ^b	35.3 ^c	30.1 ^c	25.5 ^c
	T4	1.63 ^c _d	1.30 ^{cd}	12.3 ^a _b	10.9 ^a _b	149.2 ^{bc}	654.1 ^d	1.55 ^{bc} _d	3.07 ^{bc} _d	16.9 ^{ab}	13.0 ^{bc}	1.07 ^{ab}	1.06 ^{abc}	12.1 ^{bc} _d	10.5 ^c	13.4 ^{bc}	33.8 ^{cd}	43.6 ^d	39.3 ^d
	T5	1.92 ^d _e	1.53 ^d	11.8 ^b	10.7 ^a _b	151.7 ^{bc}	669.2 ^e	1.82 ^{cd}	3.37 ^{cde}	16.2 ^{bc}	12.0 ^{cd}	1.06 ^{ab}	1.05 ^{bc} _d	14.5 ^{cde}	13.6 ^d	11.5	31.4 ^d	45.2 ^d	39.8 ^d
	T6	2.04 ^e	1.58 ^d	9.4 ^c	9.7 ^{bc}	154.4 ^c	677.7 ^f	2.03 ^d	3.49 ^{de}	15.7 ^{cd}	11.5 ^d	1.05 ^{bc}	1.04 ^{cd}	14.8 ^{de}	15.8 ^{de}	10.6	28.9 ^{de}	44.7 ^d	39.6 ^d
	T7	2.20 ^e	1.65 ^d	9.3 ^c	8.3 ^c	157.8 ^c	698.8 ^g	2.07 ^d	3.75 ^e	15.0 ^d	11.0 ^d	1.04 ^c	1.04 ^d	15.3 ^e	16.8 ^e	9.3	28.0 ^e	44.4 ^d	39.3 ^d
	Mean	1.50	1.16	11.9	10.6	147.4	649.3	1.63	3.16	16.5	12.8	1.06	1.06	12.2	11.1	13.3	34.0	35.3	31.2
Naraingarh	T1	0.52 ^a	0.26 ^a	14.0 ^a	11.8 ^a	136.4 ^a	604.8 ^a	1.20 ^a	2.63 ^a	17.9 ^a	14.5 ^a	1.08 ^a	1.07 ^a	5.6 ^a	4.5 ^a	17.2 ^a	41.3 ^a	16.9 ^a	15.5 ^a
	T2	0.90 ^b	0.48 ^a	13.4 ^a _b	11.6 ^a	139.4 ^a	614.3 ^b	1.34 ^{ab}	2.96 ^b	17.4 ^{ab}	13.9 ^a _b	1.07 ^{ab}	1.07 ^{ab}	10.2 ^b	8.6 ^{abc}	15.4 ^{ab}	38.0 ^a _b	22.6 ^b	19.2 ^b
	T3	1.37 ^c	0.85 ^b	12.4 ^b	11.2 ^a _b	143.1 ^a _b	634.6 ^c	1.41 ^{ab}	3.11 ^{bc}	17.1 ^{ab} _c	13.6 ^a _b	1.07 ^{ab}	1.06 ^{abc}	12.2 ^{bc}	9.4 ^{bc}	14.3 ^{abc}	35.5 ^{bc}	29.1 ^c	28.2 ^c
	T4	1.97 ^d	1.46 ^c	12.4 ^b	11.1 ^a _b	147.0 ^{bc}	645.0 ^d	1.43 ^b	3.24 ^{cd}	16.8 ^{bc}	12.9 ^{bc}	1.07 ^{ab} _c	1.06 ^{abc}	12.4 ^{bc}	11.5 ^{bc} _d	13.2 ^{bc} _d	32.8 ^{cd}	42.6 ^d	38.5 ^d
	T5	2.17 ^d _e	1.56 ^c	11.3 ^c	10.4 ^{bc}	151.5 ^{cd}	658.1 ^e	1.56 ^{bc}	3.24 ^{cd}	16.3 ^{cd}	12.1 ^{cd}	1.06 ^{ab} _c	1.05 ^{bc} _d	13.7 ^{bc}	12.3 ^{bc} _d	11.4 ^{cd}	30.3 ^{de}	44.0 ^d	40.5 ^d
	T6	2.20 ^d _e	1.69 ^c	9.5 ^d	9.5 ^{cd}	155.4 ^d	678.5 ^f	1.72 ^{cd}	3.40 ^{de}	16.0 ^{cd}	11.8 ^d	1.05 ^{cd}	1.04 ^{cd}	14.3 ^{bc}	14.3 ^d	10.8 ^{cd}	28.8 ^e	43.1 ^d	39.1 ^d
	T7	2.24 ^e	1.69 ^c	9.1 ^d	8.9 ^d	155.9 ^d	695.3 ^g	1.94 ^d	3.58 ^e	15.5 ^d	11.5 ^d	1.04 ^d	1.04 ^d	15.8 ^c	15.2 ^d	9.6 ^d	27.4 ^e	42.1 ^d	37.6 ^d
	Mean	1.62	1.14	11.7	10.6	146.9	647.2	1.51	3.17	16.7	12.9	1.06	1.06	12.0	10.8	13.1	33.4	34.3	31.2

Specific gravity is a critical quality parameter in potatoes, influencing their suitability for various applications, particularly in food processing. Potatoes with higher specific gravity typically have firmer textures and lower moisture

content, making them ideal for products such as fries and chips, where these traits enhance product quality (Islam *et al.*, 2022) [23]. Additionally, specific gravity provides insights into the potato's physical characteristics, including

density and susceptibility to mechanical damage, which affect storage stability and handling. For the PP102 variety, the highest specific gravity was recorded in T1 (1.08), comparable to T5 (1.06) at both L1 and L2. In the Kufri Pukhraj variety, the maximum specific gravity was observed in T1 (1.07), which was similar to T4 (1.06) as outlined in Table 2.

Crude proteins, composed of amino acid chains, are essential for growth, repair, and maintenance in living organisms. They play vital roles in metabolism, cellular function, and are key components in animal feed and human nutrition. Crude proteins are categorized into structural proteins, enzymes, and transport proteins, each supporting various biochemical processes (Wu, 2010) [73]. Assessing crude protein content is crucial for livestock production, human health, and agricultural practices aimed at enhancing food security and sustainability (Wu *et al.*, 2014) [74]. PP102 outyielded Kufri Pukhraj in terms of total crude proteins. The highest crude protein content in the PP102 variety was observed in T7 (15.3% and 15.8%), significantly differing from other treatments at both L1 and L2. For Kufri Pukhraj, T7 recorded the highest crude protein content (16.8% and 15.2%), comparable to T2 (7.4% and 8.6%) at both stages.

Vitamin C (ascorbic acid) is an essential nutrient in potatoes, playing a critical role in collagen synthesis, immune function, and iron absorption. As a powerful antioxidant, it helps neutralize reactive oxygen species (ROS), protecting cells from oxidative stress (Sami *et al.*, 2024; Pehlivan, 2017) [53, 46]. Potatoes, especially when consumed with their skins, are a valuable source of vitamin C, which contributes to overall health. The vitamin C content is influenced by factors such as storage conditions,

cooking methods, and cultivar (Love and Pavék, 2008) [33]. Among both the varieties, Kufri Pukhraj contains higher amount of vitamin C as affected by different N levels. The highest vitamin C content in PP102 was recorded in treatment T1 (17.9 mg/100g and 17.2 mg/100g), which was comparable to T2 (15.9 mg/100g) and T3 (14.3 mg/100g) at L1 and L2. For Kufri Pukhraj, T1 exhibited the highest content (42.56 mg/100g and 41.3 mg/100g), significantly differing from other treatments at L1 but comparable to T2 (38.0 mg/100g) at L2.

Tuber yield in potatoes is primarily governed by genetic potential, which dictates tuber size, number, and quality (Nigussie *et al.*, 2023) [43]. Nitrogen fertilizer application significantly influences vegetative growth and tuber development, with optimal nitrogen levels enhancing photosynthesis, root expansion, and tuber growth. Moderate nitrogen application typically results in the highest yield, as insufficient nitrogen limits growth, while excessive nitrogen promotes excessive foliage at the cost of tuber development (Ahmed *et al.*, 2009) [4]. Although soil type and weather conditions also affect yield, the interplay between genetic potential and nitrogen management is crucial for maximizing productivity (Samson *et al.*, 2019) [54]. Effective nitrogen use helps realize the plant's genetic potential, improving yield while minimizing nutrient waste and environmental impact (Hirel *et al.*, 2011) [21]. The highest tuber yield for PP102 was observed in treatment T5 (45.2 t ha⁻¹ and 44.0 t ha⁻¹), statistically comparable to T4 (43.6 t ha⁻¹ and 42.6 t ha⁻¹) at both L1 and L2. For Kufri Pukhraj, the maximum yield was in T5 (39.8 t ha⁻¹ and 40.5 t ha⁻¹), comparable to T4 (38.5 t ha⁻¹) at both L1 and L2, as summarized in Table 2.

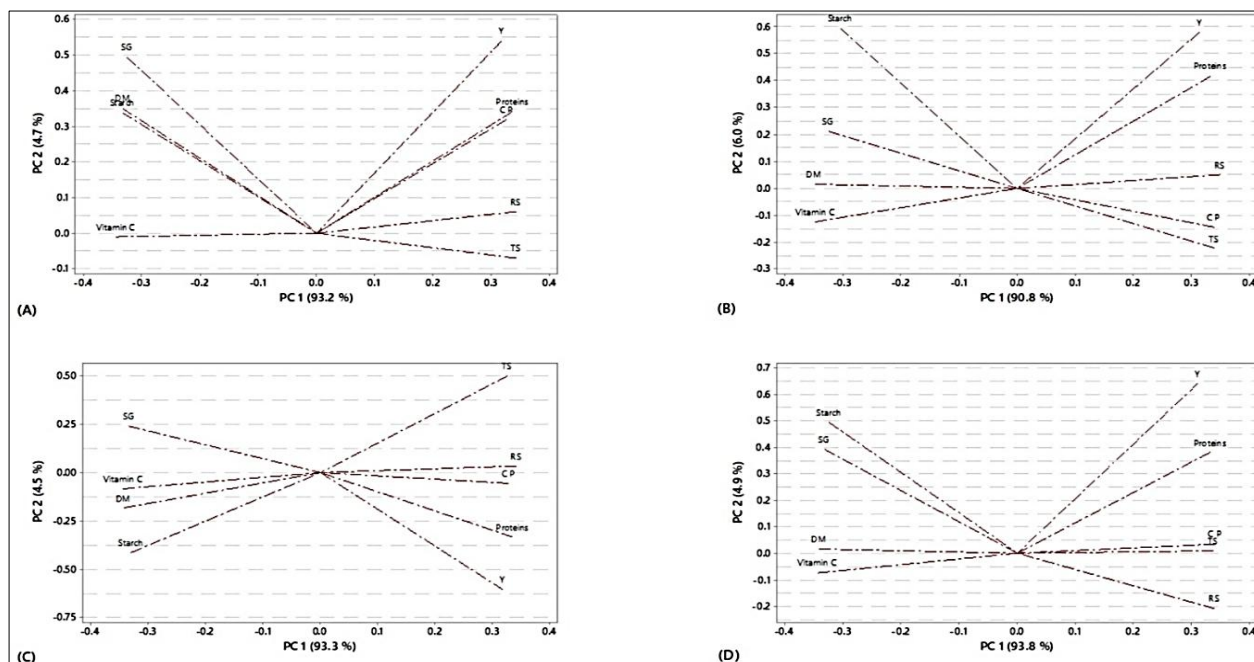


Fig 1: Pca biplot of pp102 (a, c) and kufri pukhraj (b, d) across ludhiana (a, b) and naraingarh (c, d) based on biochemical parameters and tuber yield

In the evaluation of the primary differences in the quality of various potato varieties, a thorough, systematic, and scientific assessment of multiple nutritional components should be conducted. Principal Component Analysis (PCA) serves as an effective method for such comprehensive evaluation. This study analyzed nine factors protein, starch, reducing sugars, total sugars, dry matter, specific gravity,

crude protein, vitamin C, and tuber yield across two potato varieties. The information conveyed by each principal component (PC) was quantified based on the cumulative variance. The PCA score plot (Fig. 1) illustrates the results for two potato varieties at two distinct locations.

For the PP102 variety at Ludhiana, the first two principal components explained 97.9% of the total variance (93.2%

for PC1 and 4.7% for PC2). Similarly, for variety B, the first two principal components explained 96.8% of the total variance (90.8% for PC1 and 6% for PC2). A similar trend was observed for both varieties at Naraingarh. These findings suggest that the first two principal components account for the majority of the information associated with

all the variables. As depicted in Fig. 1, it is apparent that protein, reducing sugars (RS), total sugars (TS), crude protein (CP), and tuber yield (Y) exhibit a positive correlation with PC1, whereas vitamin C, starch, dry matter (DM) and specific gravity (SG) demonstrate a negative correlation with PC1 for both varieties at both locations.

Table 3: Nitrogen content (%) and shoot biomass (t ha⁻¹) of PP102 and Kufri Pukhraj at weekly intervals from 28 to 63 DAS as affected by different levels of nitrogen at Ludhiana and Naraingarh.

	Treatments	% N												Shoot biomass (t ha ⁻¹)											
		28 DAS		35 DAS		42 DAS		49 DAS		56 DAS		63 DAS		28 DAS		35 DAS		42 DAS		49 DAS		56 DAS		63 DAS	
		V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2
Ludhiana	T1	2.20 ^a	1.95 ^a	1.97 ^a	1.77 ^a	1.83 ^a	1.63 ^a	1.48 ^a	1.23 ^a	1.43 ^a	1.12 ^a	1.13 ^a	0.99 ^a	0.403 ^a	0.38 ^a	0.84 ^a	0.611 ^a	1.43 ^a	1.12 ^a	1.92 ^a	1.81 ^a	2.26 ^a	2.24 ^a	2.74 ^a	2.37 ^a
	T2	2.99 ^a	2.09 ^a	2.77 ^a	2.01 ^a	2.63 ^a	2.09 ^a	2.14 ^a	1.94 ^a	2.00 ^a	1.85 ^a	1.71 ^a	1.64 ^a	0.472 ^a	0.41 ^a	0.95 ^a	0.806 ^a	1.71 ^a	1.17 ^a	2.28 ^a	1.97 ^a	2.74 ^a	2.54 ^a	3.08 ^a	2.84 ^a
	T3	3.84 ^b	2.79 ^a	3.31 ^b	2.48 ^a	2.81 ^b	2.30 ^a	2.47 ^b	2.09 ^b	2.29 ^b	2.15 ^b	1.88 ^b	1.71 ^b	0.486 ^a	0.43 ^a	1.08 ^a	0.861 ^a	1.90 ^a	1.38 ^a	2.64 ^a	2.19 ^a	2.94 ^a	2.65 ^a	3.18 ^a	3.11 ^a
	T4	3.91 ^b	3.09 ^b	3.37 ^b	2.97 ^b	3.09 ^b	2.65 ^b	2.57 ^b	2.39 ^b	2.36 ^b	2.29 ^b	2.05 ^b	1.75 ^b	0.528 ^a	0.51 ^a	1.10 ^a	0.931 ^a	2.28 ^a	1.68 ^a	3.13 ^a	2.67 ^c	3.35 ^b	3.10 ^b	3.57 ^b	3.45 ^b
	T5	3.95 ^b	3.56 ^c	3.49 ^b	3.33 ^c	3.28 ^b	2.97 ^c	2.59 ^b	2.51 ^b	2.44 ^b	2.37 ^b	2.19 ^b	1.92 ^b	0.570 ^a	0.52 ^a	1.26 ^b	0.986 ^a	2.49 ^c	1.93 ^b	3.32 ^a	2.73 ^c	3.66 ^c	3.39 ^c	3.72 ^c	3.66 ^c
	T6	4.05 ^c	3.63 ^d	3.84 ^b	3.41 ^c	3.41 ^b	3.25 ^d	2.89 ^b	2.77 ^b	2.67 ^b	2.43 ^b	2.31 ^b	2.04 ^b	0.639 ^a	0.54 ^a	1.38 ^c	1.10 ^{bc}	2.79 ^d	2.58 ^c	3.49 ^{cd}	3.08 ^{de}	3.79 ^d	3.62 ^d	3.87 ^c	3.80 ^c
	T7	4.45 ^c	3.87 ^d	4.03 ^c	3.43 ^c	3.83 ^c	3.27 ^d	3.01 ^b	2.85 ^c	2.72 ^b	2.55 ^b	2.37 ^b	2.21 ^b	0.708 ^c	0.61 ^a	1.43 ^c	1.18 ^c	3.24 ^e	2.93 ^c	3.72 ^d	3.64 ^e	3.89 ^d	3.89 ^d	3.99 ^c	3.86 ^c
	Mean	3.63	3.00	3.25	2.77	2.98	2.60	2.45	2.25	2.27	2.11	1.95	1.75	0.544	0.491	1.15	0.925	2.26	1.83	2.93	2.58	3.23	3.06	3.45	3.30
Naraingarh	T1	2.31 ^a	2.00 ^a	2.10 ^a	1.84 ^a	1.87 ^a	1.69 ^a	1.69 ^a	1.48 ^a	1.41 ^a	1.31 ^a	1.29 ^a	1.03 ^a	0.373 ^a	0.34 ^a	0.87 ^a	0.643 ^a	1.34 ^a	1.18 ^a	1.95 ^a	1.68 ^a	2.16 ^a	2.15 ^a	2.32 ^a	2.16 ^a
	T2	2.79 ^a	2.68 ^a	2.55 ^a	2.45 ^a	2.35 ^a	2.21 ^a	2.15 ^a	2.05 ^a	2.01 ^a	1.87 ^a	1.84 ^a	1.45 ^a	0.450 ^a	0.40 ^a	0.91 ^a	0.787 ^a	1.51 ^a	1.36 ^a	2.16 ^a	1.84 ^a	2.30 ^a	2.22 ^a	2.50 ^a	2.27 ^a
	T3	3.20 ^a	2.97 ^a	2.97 ^a	2.63 ^a	2.65 ^a	2.41 ^a	2.51 ^a	2.25 ^a	2.39 ^a	2.08 ^a	1.93 ^a	1.75 ^a	0.525 ^a	0.48 ^a	1.07 ^a	0.928 ^a	1.86 ^a	1.62 ^a	2.36 ^a	2.26 ^a	2.64 ^a	2.76 ^a	2.84 ^a	2.93 ^a
	T4	3.40 ^b	3.11 ^b	3.17 ^b	2.87 ^b	2.93 ^b	2.63 ^b	2.81 ^b	2.43 ^b	2.63 ^b	2.11 ^b	2.09 ^b	1.85 ^b	0.584 ^a	0.52 ^a	1.23 ^b	1.07 ^{bc}	2.27 ^c	1.96 ^b	2.78 ^a	2.46 ^b	3.02 ^b	3.04 ^b	3.20 ^b	3.10 ^b
	T5	3.81 ^c	3.26 ^b	3.57 ^b	3.13 ^c	3.21 ^b	2.81 ^b	2.92 ^c	2.48 ^b	2.69 ^b	2.27 ^b	2.32 ^b	2.03 ^b	0.611 ^a	0.61 ^a	1.36 ^c	1.16 ^{cd}	2.46 ^d	2.26 ^c	3.33 ^a	2.99 ^c	3.58 ^c	3.49 ^c	3.86 ^c	3.70 ^c
	T6	4.16 ^d	3.52 ^b	3.84 ^b	3.28 ^c	3.45 ^b	2.99 ^c	3.05 ^b	2.69 ^b	2.79 ^b	2.45 ^b	2.41 ^b	2.12 ^b	0.640 ^a	0.62 ^a	1.51 ^d	1.29 ^{de}	2.80 ^f	2.56 ^d	3.56 ^b	3.03 ^c	3.86 ^d	3.55 ^d	4.09 ^d	4.04 ^d
	T7	4.48 ^e	3.73 ^c	4.15 ^d	3.51 ^d	3.81 ^c	3.13 ^d	3.27 ^c	2.88 ^c	2.97 ^c	2.51 ^b	2.47 ^b	2.25 ^b	0.713 ^e	0.64 ^a	1.68 ^e	1.45 ^e	3.00 ^f	2.75 ^e	4.21 ^e	3.32 ^d	4.29 ^e	3.83 ^d	4.52 ^d	4.14 ^e
	Mean	3.45	3.04	3.19	2.82	2.90	2.55	2.63	2.32	2.41	2.09	2.05	1.78	0.556	0.520	1.23	1.05	2.18	1.96	2.91	2.51	3.12	3.01	3.33	3.19

Nitrogen is vital for potato growth, influencing both vegetative and tuber development. Absorbed as nitrate or ammonium, N supports early foliage and stem growth, then translocate to tubers to enhance enlargement. N levels increase during bulking but stabilize as the plant matures. Proper nitrogen management is crucial insufficient N limits growth and yield, while excess promotes vegetative growth at the expense of tuber formation and increases disease risk. Monitoring N levels throughout the growing season is key to optimizing yield and quality (Muleta and Aga, 2019; Alva, 2004) [38, 6]. The N content of both the varieties decreases after every week from 28 to 63 DAS. PP102 accumulates high N content in aerial parts as compared to that of Kufri Pukhraj at every sampling stage at both the locations.

Shoot biomass in potatoes plays a pivotal role in growth and tuber development by facilitating photosynthesis, which is essential for energy production. Comprising primarily of leaves, stems, and branches, shoot biomass is significantly

influenced by nutrient availability, particularly nitrogen (Evans, 2013) [16]. During the early growth phase, rapid biomass accumulation occurs in the leaves, enhancing photosynthetic efficiency. As the plant progresses toward maturity, biomass accumulation slows, with a shift in resource allocation towards tuber development. Peak shoot biomass is typically observed prior to tuber bulking, after which energy is redirected to tuber growth. Optimal nutrient management, particularly with regard to nitrogen, is critical for maximizing shoot biomass, improving photosynthetic efficiency, and enhancing overall yield (Wang *et al.*, 2024) [71]. Initially, shoot biomass increases exponentially until the tuber initiation stage, after which the rate of increase slows. Notably, the cultivar PP102 demonstrates higher biomass accumulation compared to Kufri Pukhraj at all stages (Fig. 2). Additionally, nitrogen application has a positive effect on shoot biomass accumulation in both varieties, with higher N levels correlating with increased biomass production.

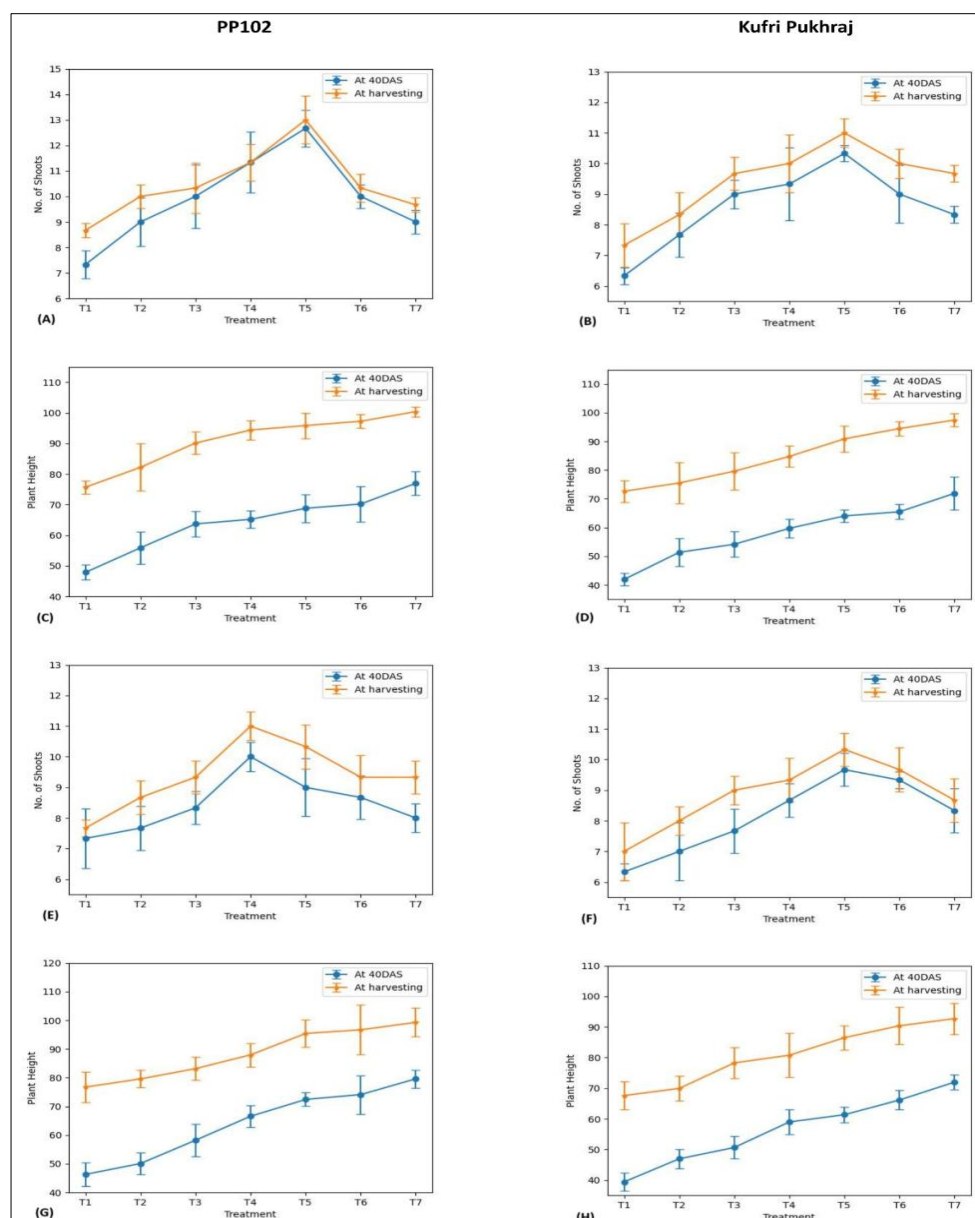


Fig 2: Plant height (in cm) and number of shoots of pp102 (a, c, e, g) and kufri pukhraj (b, d, f, h) as affected by different levels of nitrogen at ludhiana (a, b, c, d) and naraingarh (e, f, g, h)

Plant height in potatoes serves as a critical indicator of vegetative growth, influenced by genetic potential, nutrient availability, and environmental factors (Miao *et al.*, 2024) [36]. During early growth stages, plant height increases as shoots develop, with nitrogen playing a key role in promoting stem and leaf elongation. Peak plant height is typically reached just before or during tuber formation, after which growth rate slows (Guo *et al.*, 2018) [19]. While excessive nitrogen can lead to excessive vegetative growth and reduced tuber yield, optimal nitrogen application balances height growth and tuber development. Monitoring plant height throughout the growing season provides valuable insights for effective management strategies aimed at maximizing yield. The cultivar PP102 demonstrated greater plant height compared to Kufri Pukhraj at both 40 days after planting (DAS) and at harvest. The effect of nitrogen on plant height was significant, with increasing nitrogen doses resulting in taller plants. Specifically, the plant height of PP102 reached 76.9 cm and 79.6 cm at 40 DAS and 93.8 cm and 99.3 cm at harvest, while Kufri Pukhraj showed heights of 71.9 cm and 72 cm at 40 DAS and 96.1 cm and 92.1 cm at harvest, as depicted in Fig. 2.

The number of shoots in potato plants is a critical factor for both growth and tuber production, as each shoot has the potential to contribute to tuber formation (Almekinders and Struik, 1996) [5]. Shoot development primarily occurs during the early vegetative stage and is influenced by genetic factors, nutrient availability, and environmental conditions. Nitrogen plays a pivotal role in promoting shoot formation. Thus, optimizing shoot numbers requires careful nitrogen management. Monitoring shoot count can help refine nutrient applications to maximize tuber yield (Muleta and Aga, 2019) [38]. The cultivar PP102 exhibited a significantly higher number of shoots compared to Kufri Pukhraj. Additionally, the application of optimal nitrogen levels resulted in the highest shoot numbers for both varieties, with the application of 250 kg N ha⁻¹ (T5) yielding the maximum shoot count, shown in Fig. 2.

4. Discussion

Proteins are vital for plant structure, enzyme activity, and growth regulation. Nitrogen, essential for amino acids, supports protein biosynthesis, with adequate nitrogen promoting growth and protein production, while its

deficiency limits development. The increase in protein content in potato tubers can be attributed to a rise in amino acid levels and peptide bonds between molecules. Similar effects were observed in barley and wheat with higher nitrogen (Yadav *et al.*, 2020; Abedi *et al.*, 2011) ^[75, 1]. Variations in protein content among cultivars are genetic, as noted by Marwaha, (2003) ^[35] and Lehesranta, (2005) ^[30].

Excessive nitrogen application reduces starch content by reallocating resources from starch synthesis to vegetative growth, while promoting biomass production at the expense of tuber development (Sun *et al.*, 2019) ^[65]. However, it increases total sugar content due to elevated amino acid levels resulting from nitrogen application (Sandhu *et al.*, 2010) ^[55]. Hameed *et al.*, (2018) ^[20] observed that the sugar content in potato tubers is a genetically inherited trait, which can be influenced by the specific cultivar. The disparity in sugar content between these two varieties can be attributed to their differing genetic compositions, which govern their carbohydrate metabolism, including the synthesis of sugars. Lauret *et al.*, (2023) ^[28] also observed variations in starch content across different potato varieties.

Excessive nitrogen application significantly impacts potato production, as it reduces both dry matter content and specific gravity of tubers. Increased nitrogen elevates moisture levels, diluting the dry matter proportion, which has been observed in previous studies (O'Beirne and Cassidy, 1990; Ahmed *et al.*, 2009) ^[44, 4]. This higher nitrogen input also leads to a decline in specific gravity, a trend consistent with findings by Ahmed *et al.*, (2009) and Westermann *et al.*, (1994) ^[72]. The reduction in specific gravity is closely associated with the decrease in dry matter content, as a strong positive correlation between dry matter and specific gravity has been established (Schipper, 1976) ^[57]. The reduced tuber yield in the no-N treatment highlights the insufficient native nitrogen for optimal growth. *Tuber yield* significantly declined beyond a certain nitrogen level, likely due to excessive nitrogen, as observed by Oliveira, (2000) ^[45]. Sriom *et al.*, (2017) ^[63] found a positive correlation between nitrogen rate and tuber number up to an optimal level, while Zvomuya and Rosen, (2001) ^[77] noted similar trends, with effects plateauing at this level. Ayyub *et al.*, (2019) ^[7] reported reduced stem number with nitrogen exceeding a certain threshold, emphasizing the need for balanced nutrient management. The higher yield in PP102 is attributed to improved traits such as stem count, plant height, and tuber weight, which were more prominent than in Kufri Pukhraj. This supports Fawtaw *et al.*, (2019), who linked better yield to enhanced agronomic traits.

Plant nitrogen content varies significantly across treatments and varieties, primarily influenced by increased nitrogen application, which affects growth parameters and assimilation rates. Zedan, (2011) ^[76] found that higher nutrient levels lead to increased assimilation in both reproductive and vegetative tissues. Differences in nitrogen uptake among varieties are attributed to genetic variability and morphological factors (Horváth *et al.*, 2019) ^[22]. The increase in shoot biomass follows a linear trend, likely due to changes in plant growth and nutrient distribution, with nutrients being redirected from aboveground parts to areas with higher demand. Villa *et al.*, (2020) ^[69] similarly reported increased shoot biomass with rising nitrogen levels in potato. Nitrogen application significantly impacts all growth stages by enhancing nutrient availability, promoting greater biomass accumulation, a finding also noted by

Belanger *et al.*, (2002) ^[9]. Excessive nitrogen application can disrupt the balance of key plant hormones, particularly auxin and cytokinin, which are critical for promoting shoot growth. Ayyub *et al.*, (2019) ^[7] observed that excessive nitrogen leads to nutrient imbalances, negatively affecting shoot development and overall plant health. This can result in reduced shoot production as the plant struggles to allocate resources efficiently, and may cause physiological stress, making the plant more susceptible to environmental stressors (Abro, 2013) ^[2], which further diminishes shoot number. On the other hand, the increase in plant height observed with nitrogen treatments can be attributed to an adequate nitrogen supply, which promotes enhanced cell elongation and division, leading to more vigorous shoot growth. These findings align with those of Ayyub *et al.*, (2019) and Ahmed *et al.*, (2009) ^[4], who similarly reported an increase in plant height with higher nitrogen application.

Conclusion

In conclusion, nitrogen fertilization plays a crucial role in enhancing the vegetative growth of potato plants, as evidenced by increased plant height and shoot biomass. It also contributes positively to nitrogen content within the plants, promoting better overall growth. However, excessive nitrogen application can have detrimental effects on certain biochemical parameters, such as dry matter, specific gravity, starch content, and vitamin C, while leading to increases in protein content, reducing sugars, and total sugars. These findings highlight the importance of optimizing nitrogen levels to maximize both the yield and nutritional quality of potato crops. Therefore, balanced nitrogen management is essential to achieve sustainable potato production, ensuring high crop yield without compromising tuber quality and nutritional value.

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Author Contributions

Abhishek Grover: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - Original Draft. **A.A.S. Gill:** Conceptualization, Supervision. **Monika Mahajan:** Writing - Review & Editing, Resources. **Satpal Sharma:** Resources.

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