AGRO-PHYSIOLOGICAL AND TRANSCRIPTOME ANALYSIS OF POTATO VARIETIES UNDER DIFFERENT NITROGEN CONDITIONS IN AEROPONICS

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY
in

Biotechnology

By

Rasna Zinta
Registration Number: 12109326

Supervised By

Dr. Umesh Goutam (14691)

Professor

Department of Molecular Biology and Genetic Engineering, School of Bioengineering and Biosciences, LPU

Co-Supervised by

Dr. Jagesh Kumar Tiwari

Principal Scientist

ICAR-IIVR, Varanasi

Dr. Tanuja Buckseth

Senior Scientist, ICAR-CPRI, Shimla

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2025

DECLARATION

I, hereby declare that the presented work in the thesis entitled "Agro-physiological and transcriptome analysis of potato varieties under different nitrogen conditions in aeroponics" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of Dr. Umesh Goutam, working as Head of Department, in the Department of Molecular Biology and Genetic Engineering of Lovely Professional University, Punjab, India and co-supervision of Dr. Jagesh Kumar Tiwari & Dr. Tanuja Buckseth of Division of Crop Improvement & Seed Technology, ICAR-CPRI, Shimla. In keeping with the general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigators. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

(Signature of Scholar)

Rasna Zinta
Registration No.: 12109326
Department of Biotechnology /
School of Bioengineering and Biosciences
Lovely Professional University, Punjab,
India

CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled “Agro-physiological and transcriptome analysis of potato varieties under different nitrogen conditions in aeroponics” submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the Department of Biotechnology School of Bioengineering and Biosciences, is a research work carried out by **Rasna Zinta (Reg. No. 12109326),** is bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

(Signature of Supervisor)
Dr. Umesh Goutam
Professor and Head
Molecular Biology and
Genetic Engineering
School of Bioengineering
and Biosciences
Lovely Professional University
Phagwara, Punjab (INDIA) -144411

(Signature of Co-supervisor)
Dr. Jagesh Kumar Tiwari
Principal Scientist
Division of Vegetable Improvement
ICAR-IIVR
Varanasi, UP (INDIA) -221305

(Signature of Additional Co-supervisor)
Dr. Tanuja Buckseth
Senior Scientist
Division of CI & ST
ICAR-CPRI
Shimla, HP (INDIA) -171001

ABSTRACT

Potatoes (*Solanum tuberosum* L.), the third most important food crop worldwide, are highly dependent on nitrogen (N) for optimal plant growth, tuber yield, and quality. N is a key nutrient that supports photosynthesis, root development, and the synthesis of essential proteins, which directly impacts the size and quality of the tubers. In India, the second-largest producer of potatoes globally after China, potato cultivation plays a significant role in food security and the agricultural economy. The country's diverse climate conditions and varying soil types offer both challenges and opportunities for improving potato productivity through efficient nitrogen management. Optimizing nitrogen use can enhance crop yield, reduce environmental impacts, and ensure better tuber quality, making it a critical factor for sustainable potato farming in India. The approach of Nitrogen Use Efficiency (NUE) in potatoes is crucial for the improvement of crop yield and reduction in the detrimental effects caused by nitrogenous fertilizers. Potatoes are a nitrogen-demanding crop, requiring substantial amounts of nitrogen fertilizer (150–300 kg N per hectare) to achieve high tuber yields (30–50 tonnes per hectare). However, only 40–50% of the total nitrogen is absorbed by the plant; the remainder is lost to the environment. This contributes to the emission of N oxides and pollution caused by the leaching of nitrates resulting in the emission of greenhouse gases, which further contaminate air quality and the fertility of the soil, consequently, there is a need to increase the NUE in order to boost productivity, reduce the cost of fertilizers and mitigate the leaching and greenhouse gases problems associated with N in the soils. This study involves physiological, genetic, and agronomic aspects of NUE in potatoes, with a focus on mechanisms related to nitrogen uptake, assimilation, and remobilization within the plant. Certain NUE traits, such as root architecture, nitrogen transporter efficiency, and metabolic pathways have been defined indicating that there are prospects for breeding potato varieties that consume less nitrogen. Besides these, agronomic practices such as fertilizer management, crop rotation, and soil health also matter in productive efficiency. Now that molecular breeding and genomic techniques are being used, the creation of such cultivars with higher NUE becomes possible which on one hand can improve yield while at the same time, nitrogen fertilization usage will be reduced. Tuber is the commercially significant underground plant portion of a potato, and for its growth and development, roots play an essential role for absorbing nutrients and water. Plant anchoring, nutrition, and water uptake, and environmental

advantages including carbon sequestration and decreased soil erosion are all greatly aided by root architecture. Not enough is known about root system architecture (RSA) in potatoes, in contrast to the substantial study on rice, wheat, and maize. A deep-rooted potato ideotype would be more suitable for deeper soils, whereas a shallow-rooted ideotype can efficiently absorb water and nutrients from the upper soil layers, as nitrogen compounds are mobile and tend to leach downward. A potential and underutilized approach to address the pressing demand for resource-efficient and climate-resilient crops in global agriculture is profiling RSA and its application. Therefore, using contemporary genomics technologies to create abiotic stress-tolerant cultivars requires a fresh focus on root architecture.

A soil-free, mist-driven nutrient delivery system, aeroponics, in which a plant, along with its root is hung in the air, has been used globally to produce high-quality seed potatoes (mini tubers). This technology has also been known for various applications in the fields of physiology, genomics, breeding, and also for demonstrating transcriptome analysis under N stress. Precision phenotyping of potatoes using aeroponic technology has been demonstrated and there is a great deal of promise for further research. Utilizing germplasm and variety potential to improve NUE in potatoes, especially root phenotyping, is now receiving attention. More than 70 potato varieties have been developed in India since the 1950s to be grown in the various climate zones of the nation. The lack of knowledge and literature regarding Indian potato cultivars' RSA, NUE, and yield-contributing characteristics, however, makes it difficult to breed for nutrient use efficiency. Therefore, this study sought to analyze the variation among 56 available potato varieties through detailed phenotyping in a soil-free aeroponic system under both high and low nitrogen conditions. Findings of this study revealed a diverse range of responses in varieties for plant biomass, root morphology, yield-contributing traits, and NUE variables. This diversity was consistent across old to new varieties (year of release: 1960s to 2020), and highlights the use of diverse source in breeding programs. High-performing and widely adopted cultivars exhibited superior root system architecture, increased biomass accumulation, enhanced tuber productivity, and greater nitrogen use efficiency, underscoring their agronomic advantage. The information gathered from this study is valuable for the development of new potato varieties. Furthermore, assessing potato varieties under varying nitrogen levels (low and high) in both aeroponic and field environments offers valuable insights for optimizing performance in low-input agricultural systems.

Further, RNA-seq analysis was also done for the identification of genes responsible for the tuber growth under the aeroponics system and finally, validation of a few candidate genes was carried out through real-time qPCR analysis. Several candidate genes that are probably involved in producing high tuber yields in aeroponics under high nitrogen levels have been found by transcriptomic profiling. These genes include a nitrate transporter, glutamine synthetase, aminotransferase, GDSL esterase/lipase, sucrose synthase, UDP-glycosyltransferases, osmotin, xyloglucan endotransglucosylase/hydrolase, laccases, glutaredoxin, and several transcription factors (like BTB/POZ, AP2/ERF, and MYB).

Keywords: Transcriptome sequencing, *Solanum tuberosum* L., Aeroponics, Genes, Plant phenotype, Root system architecture, Agronomic traits, Tuber yield traits, Nitrogen Use Efficiency, qRT-PCR.

Dedicated To

my respected mentor and co-supervisor

Dr. Jagesh Kumar Tiwari

& my beloved parents

ACKNOWLEDGEMENT

It is a genuine pleasure to express my deep sense of thanks and gratitude to my guide and supervisor **Dr. Umesh Goutam**, Professor, HOD, Genetic engineering and Molecular Biology, Lovely professional University, Phagwara. His dedication and keen interest above all his overwhelming attitude to help his students had been solely and mainly responsible for completing my work. His timely advice, meticulous scrutiny, scholarly advice and scientific approach have helped me to a very great extent to accomplish this task.

I would like to express my sincere gratitude to my co-supervisor **Dr. Jagesh Kumar Tiwari,** Principal Scientist, ICAR-Central Potato Research Institute, Shimla, now transferred to ICAR-IIVR, Varanasi, for his continuous support of my Ph.D. study and research, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research, without him, this venture would'nt have been possible, I owe it all to him and would like to dedicate this dissertation as an expression of gratitude and respect to him.

I extend my gratitude to my additional co-supervisor **Dr. Tanuja Buckseth,** Senior Scientist and Incharge Aeroponics unit; **Dr. Ajay Kumar Thakur,** Principal Scientist and Incharge Cell and Molecular Biology Lab (CMBL); and **Dr. Vinod Kapoor,** Acting Head and Principal Scientist; Division of CI & ST, ICAR-Central Potato Research Institute, Shimla for their input and valuable discussions during my research tenure. I would also like to thank **Dr. Brajesh Singh,** Director, ICAR-Central Potato Research Institute, Shimla, for all the support, guidance and facilities provided at the institute.

I thank my fellow lab mates and friends of ICAR-Central Potato Research Institute, Shimla: Dr. Bhawna Dipta, Dr. Neha Sharma, Miss Vineeta Sharma, Dr. Neha Salaria, Dr. Kanika Thakur, Mrs. Reena, Dr. Suhani, Mrs. Deepali, Dr. Ashwani for the stimulating discussions and encouragement and trainees Miss Arshia Prashar, Miss Muskaan Thakur and Miss Srishti Singh for their assistance during the course of my research. I would also like to thank the technical staff, especially Sh. Sheesh Ram, Sh. Neem Chand and Sh. Devraj of ICAR-Central Potato Research Institute, Shimla for their support throughout my research.

Last but not the least I would like to express my family for their everlasting

support and encouragement without which achieving my goal was not possible.

Finally, there are too many to name individually, for their enormous help, moral support, motivation and kindness. To my family and friends, you should know that your support and encouragement was worth more than I can express on paper.

(Rasna Zinta)

PREFACE

The potato (*Solanum tuberosum* L.) is cultivated all across the world, in which India ranks second after China. It ranks as the third most important food crop globally, following rice and wheat. Nitrogen Use Efficiency (NUE) in potato is a key factor in optimizing crop yield while minimizing the environmental impacts of nitrogen fertilizer use. Potatoes are a nitrogen-intensive crop, therefore improving NUE is essential for enhancing productivity, reducing fertilizer costs, and mitigating environmental issues such as nitrogen leaching and greenhouse gas emissions. Therefore, understanding the genes associated with nitrogen metabolism is crucial for improving nitrogen use efficiency (NUE) in plants. Additionally, because of their shallow root systems, which extend only 20–30 cm into the soil, these plants absorb just 40–50% of the applied nitrogen fertilizer. The remainder is either leached into groundwater, lost to the environment, or retained in the soil for use by subsequent crops. Therefore, advancing our understanding of root system architecture (RSA) in potatoes is critical and requires integrated physiological, biochemical, and molecular strategies to develop cultivars that use resources more efficiently. Investigating and applying RSA traits represents a promising yet underutilized approach to breeding climate-resilient and resource-efficient potato varieties essential for sustainable global agriculture.

The facilities provided by ICAR-Central Potato Research Institute, Shimla and the guidance of my supervisor Dr. Umesh Goutam and co-supervisors Dr. Jagesh Kumar Tiwari & Dr. Tanuja Buckseth made it possible for the achievement of the objectives.

The present research was carried out for the fulfillment of Ph.D. thesis work and proved to be a breakthrough in the discovery of key genes linked to traits influencing crop yield in potatoes under varying N supplies.

TABLE OF CONTENTS

S. NO.	TOPIC	PAGE NO
1.	Introduction	1-3
2.	Review of Literature	4-13
3.	Hypothesis	14
4.	Objectives	15
5.	Material and Methods	16-34
6.	Results and Discussion	35-156
7.	Conclusion and Future Remarks	157-158
8.	Bibliography	159-174

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
Table 5.1.	Detail of potato varieties used in this study	16
Table 5.2.	5 N Mix High N	23
Table 5.3.	0.5 N Mix Low N	24
Table 5.4.	Comparison	25
Table 6.1.	Mean performance of potato varieties for two years (pooled) data on plant biomass traits under different N treatments (high N and low N) in aeroponics	41
Table 6.2.	Mean performance of potato varieties for two years (pooled) data on tuber yield traits under different N treatments (high N and low N) in aeroponics	46
Table 6.3.	Mean performance of potato varieties for two years (pooled) data on root morphology traits under different N treatments (high N and low N) in aeroponics	49
Table 6.4.	Mean performance of potato varieties for two years (pooled) data on Nitrogen Use Efficiency (NUE) parameters under different N treatments (high N and low N) in aeroponics	54
Table 6.5.	Two-way ANOVA analysis for varieties and N treatments for pooled data of two years	59-81
Table 6.6.	RNA-seq data summary and reference mapping with the Potato genome	86
Table 6.7.	DEGs summary	87
Table 6.8.	Selected top differentially expressed genes in tuber tissues of potato varieties under high N versus low N (control) under aeroponics	88
Table 6.9.	Selected top differentially expressed genes in leaf tissues of potato varieties under high N versus low N (control) under aeroponics	91
Table 6.10.	Selected top differentially expressed genes in tuber tissues of potato varieties Kufri Pukhraj versus Kufri Jyoti (control) under different N regimes in	94

	aeroponics	
Table 6.11.	Selected top differentially expressed genes in leaf tissues of potato varieties Kufri Pukhraj versus Kufri Jyoti (control) under different N regimes in aeroponics	97
Table 6.12.	GO annotation summary	100
Table 6.13.	KEGG Annotation Statistics	101
Table 6.14.	KEGG Pathway classification	101
Table 6.15.	Validation through qRT-PCR analysis	112
Table 6.16.	RNA-seq data generation and reference mapping	116
Table 6.17.	Differentially expressed genes (DEGs) summary	117
Table 6.18.	Selected differentially expressed genes (DEGs) associated with tuber yield-related traits in tuber tissues of potato grown under aeroponics	118
Table 6.19.	Selected differentially expressed genes (DEGs) associated with tuber yield-related traits in leaf tissues of potato grown under aeroponics	123
Table 6.20.	GO annotation summary	128
Table 6.21.	KEGG Annotation Statistics	130
Table 6.22.	KEGG Pathway classification	130
Table 6.23.	Validation of selected genes through qRT-PCR analysis	132

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
Fig 1.1	Illustration of the work plan employed in the present study	3
Fig 2.1	Layout of the Aeroponic system for potato cultivation	11
Fig 2.2	Full crop growth stage of Indian potato varieties grown at the Aeroponic system installed at ICAR-CPRI, Shimla.	12
Fig. 6.1	Evaluation of potato varieties under high N and low N treatments under aeroponics (early stage)	64
Fig. 6.2	Evaluation of potato varieties under high N and low N treatments under aeroponics (full crop stage)	64
Fig. 6.3.1-6.3.17	Graphs depicting 17 agrophysiological traits of 56 potato varieties under high & low N regimes.	65-81
Fig. 6.4	Phenotypic performance of potato varieties Kufri Jyoti and Kufri Pukhraj under high N (5 mM) and low N (0.5 mM) regimes in aeroponics conditions. The arrow indicates tuber formation in these potato varieties.	103
Fig.6.5	Heat maps of the top 50 differentially expressed genes (*p* < 0.05) in tuber tissues of potato variety Kufri Pukhraj under high N (low N (control)) by RNA-seq. In a heat map, each horizontal line refers to a gene. Relatively up-regulated genes are shown in red colour, whereas down-regulated genes are shown in green colour.	104
Fig. 6.6.	Heat maps of the top 50 differentially expressed genes (*p* < 0.05) in leaf tissues of potato variety Kufri Pukhraj under high N (low N (control)) by RNA-seq. In a heat map, each horizontal line refers to a gene. Relatively up-regulated genes are shown in red colour, whereas down-regulated genes are shown in green colour.	105
Fig. 6.7.	Venn diagrams showing common genes (up-regulated and down-regulated) in potato varieties Kufri Jyoti and Kufri Pukhraj under high N versus (HN) low N (control) (LN)	106

	regimes.	
Fig. 6.8.	Venn diagrams showing common genes (up-regulated and down-regulated) between tuber and leaf tissues of Kufri Jyoti and Kufri Pukhraj.	107
Fig. 6.9.	Gene Ontology (GO) characterization for cellular component, molecular function, and biological process of up-regulated and down-regulated DEGs in potato cv. Kufri Pukhraj under high N versus low N (control).	108
Fig. 6.10.	Scatter plot and Volcano plot analysis of up-regulated and down-regulated DEGs in potato cv. Kufri Pukhraj under high N versus low N (control).	109
Fig. 6.11.	KEGG pathways categorization of up-regulated and down-regulated DEGs in potato cv. Kufri Pukhraj under high N versus low N (control).	110
Fig. 6.12.	A schematic view of differentially expressed genes likely to be involved in potato above-ground (leaf) and under-ground (tuber) plant parts for up-regulated and down-regulated genes in potato variety Kufri Pukhraj under high N for high tuber yield under aeroponics.	111
Fig. 6.13.	Phenotypic traits and tuberization trend in 50-day-old potato plants of varieties namely Kufri Frysona, Kufri Khyati, Kufri Mohan, and Kufri Sutlej (control), grown under aeroponic conditions in the Shimla hill area.	134
Fig 6.14.	Heat maps of the top 50 differentially expressed genes (*P* < 0.05) in tuber tissues of potato variety Kufri Mohan versus Kufri Sutlej (control) associated with tuber yield-related traits under aeroponics. In a heat map, each horizontal line refers to a gene. Relatively up-regulated and down-regulated genes are shown in red and green colour, respectively.	135
Fig.6.15.	Heat maps of the top 50 differentially expressed genes (*P* < 0.05) in leaf tissues of potato variety Kufri Mohan versus Kufri Sutlej (control) associated with tuber yield-related traits under aeroponics. In a heat map, each horizontal line refers to a gene. Relatively up-regulated and down-regulated genes are shown in red and	136

	green colour, respectively.	
Fig.6.16.	Heat maps of the top 50 differentially expressed genes ($P < 0.05$) in tuber tissues of potato varieties Kufri Frysona versus Kufri Sutlej (control) associated with tuber yield-related traits under aeroponics.	137
Fig. 6.17.	Heat maps of the top 50 differentially expressed genes ($P < 0.05$) in tuber tissues of potato varieties Kufri Khyati versus Kufri Sutlej (control) associated with tuber yield-related traits under aeroponics.	138
Fig. 6.18.	Heat maps of the top 50 differentially expressed genes ($P < 0.05$) in leaf tissues of potato varieties Kufri Frysona versus Kufri Sutlej (control) associated with tuber yield-related traits under aeroponics.	139
Fig. 6.19.	Heat maps of the top 50 differentially expressed genes ($P < 0.05$) in leaf tissues of potato varieties Kufri Khyati versus Kufri Sutlej (control) associated with tuber yield-related traits under aeroponics.	140
Fig. 6.20.	Venn diagram showing common differentially expressed genes (DEGs) in the tuber tissues of potato varieties *viz.* Kufri Frysona, Kufri Khyati, Kufri Mohan, and Kufri Sutlej (control) under aeroponics.	141
Fig. 6.21.	Venn diagram showing common differentially expressed genes (DEGs) in the leaf tissues of potato varieties *viz.* Kufri Frysona, Kufri Khyati, Kufri Mohan, and Kufri Sutlej (control) under aeroponics.	142
Fig. 6.22.	The Gene Ontology (GO) annotation of up-regulated and down-regulated differentially expressed genes (DEGs) in three GO domains (Cellular Component, Molecular Function, and Biological Process) in tuber tissues of Kufri Mohan versus Kufri Sutlej (control).	143
Fig. 6.23.	Scatter plot and volcano plots showing significant differentially expressed genes (DEGs) in different colour dots between Kufri Mohan versus Kufri Sutlej (control) in a) tuber tissues, b) leaf tissues. Dots in different colours represent red (up-regulated), green (down-	144

	regulated), and grey (non-significant).	
Fig. 6.24.	The KEGG pathways classification of annotated genes in leaf and tuber tissues of potato varieties *viz.* Kufri Frysona, Kufri Khyati, Kufri Mohan, and Kufri Sutlej (control) under aeroponics. X and Y axes indicate gene count and KEGG pathways, respectively.	145

Chapter-1
Introduction

CHAPTER 1 INTRODUCTION

It will be a major issue to feed the world's predicted population of more than 9 billion people by 2050. This problem is exacerbated in developing countries, where persistent soil deterioration, a scarcity of artificial fertilizers at high cost, and arable land are all severe impediments. At present, global nitrogen fertilizer usage exceeds 100 million tonnes, with root and tuber crops accounting for approximately 2.8 million tonnes of this total.

After rice and wheat, potato (*Solanum tuberosum* L.) ranks as the third most significant food crop globally in terms of human consumption. Nitrogen (N) has a major effect on crop development, tuber quantity and quality; and thus, serves as a limiting nutrient in potato cultivation. Potato in general requires a high input of nitrogen to provide a good yield; for example, for the production of 20-35 t/ha tubers, 160-240 kg N/ha is a prerequisite in the Indo-Gangetic alluvial soils (Trehan et al., 2008). Furthermore, it has a shallow-root system (20-30 cm soil depth) that is mostly grown under irrigated conditions (500-700 mm water on well-drained sandy soil), thereby promoting groundwater contamination and leaching of nitrate into the soil (NO_3^-) (Ospina et al., 2014). According to Trehan et al. (2008), a potato crop that produces 25–30 tons of tubers per hectare typically requires 120–140 kg of nitrogen per hectare from the soil. It consumes 40–50% of the nitrogen fertilizer that is given to the potato; the remainder is either maintained in the soil for use by other crops, leached into the groundwater, or lost in the environment. Mitigating the environmental impacts of nitrogen loss and the resulting financial losses necessitates enhancing nitrogen use efficiency (NUE) in potatoes, focusing particularly on root system biology.

System biology has been emphasized as a key approach for enhancing nitrogen nutrition, as plant nitrogen uptake and its regulation involve complex systems. Using the plant's principal nitrate response system, researchers have outlined some of the fundamental mechanisms of NUE. Recent developments in identifying the molecular mechanisms underlying the physiological and developmental reactions of roots to variations in N supply were assessed by Nacry et al. (2013).

More and more of these processes are being discovered, especially in the model plant *Arabidopsis*. The last ten years have seen the discovery of most of the root membrane transport proteins that control N absorption. Recent findings on molecular modulators of ammonium and nitrate sensing and signaling have revealed a significant link between nitrogen and hormone signaling pathways, alongside the identification of similar regulatory genes involved in transport and root development. To illustrate, in *Arabidopsis*, NO_3^- uptake via low-affinity transporter system (LATS) and high-affinity transporter system (HATS) activity has been associated with several NO_3^- transporter genes. Additionally, plants that experience a period of NO_3^- starvation followed by resupply exhibit a strong initial HATS activity, which is subsequently suppressed once adequate NO_3^- levels are restored. This pattern is commonly recognized as the primary response to NO_3^- (Garnett et al., 2015).

Finding NUE-associated characteristics, which are typically governed by several genes, and breeding novel nitrogen-use-efficient varieties were the primary goals of the study. Furthermore, the release and identification of cultivars with increased NUE has been hampered by the complex genetics and genotype-environment interactions. Recent advancements in high-throughput phenotyping platforms, plant physiology and genomics have led to new observations into NUE-related studies, hence providing better understanding of tools for crop improvement. Different agro-physiological and molecular studies for the improvement of nitrogen use efficiency (NUE) have been documented in cereal crops (Garnett et al., 2015). Some of them were mainly concerned with significant NUE aspects namely high-throughput phenotyping, nitrate regulation, transcription factors, microRNAs and root-based approaches (Garnett et al., 2009). The significance of various approaches for improving NUE has also been discussed for a variety of plants, including maize, wheat, rice, and *Arabidopsis*.

While some studies looked at NUE aspects at the plant level, such as genetic diversity (Zebarth et al., 2008; Ospina et al., 2014), root systems (e.g., Villordon et al., 2014; Wishart et al., 2013; White et al., 2013), and gene expression (Zebarth et al., 2011, 2012), the majority of potato studies concentrated on agronomic strategies particularly site-specific nutrient management to improve the efficiency of nitrogen fertilization (Zebarth and Rosen 2007; Vos 1997, 2009). Regarding NUE in crops like potatoes, Van Bueren and Struik (2017) documented advancements in breeding, conventional methods, and variety selection. In potatoes, N responsive gene regulatory motifs were discovered by Gálvez et al. (2016). Consequently, there is a paucity of understanding of the mechanisms at the molecular and physiological levels underpinning the metabolism of N in potatoes, which has hindered its improvement with enhanced NUE at a genetic level.

RNA-sequencing, one of the Next-Generation Sequencing (NGS) technologies, contributes to the discovery of novel transcripts/genes associated with N-responsive growth inplants (Tiwari et al., 2018). Fukushima and Kusano (2014) employed an omics approach that integrated transcript and metabolite profiling to elucidate the regulation of nitrogen metabolism, signaling, and the coordination of carbon-nitrogen metabolism in plants. Similarly, Simons et al. (2014) combined omics approaches (transcriptome, metabolome etc.) using high-quality genome-scale models with metabolic flux information under diverse nitrogen mechanisms for a better knowledge of N control in maize. An improved understanding of the physiological and biochemical background of NUE can be gained by using omics technology, especially transcriptomics, which can reveal the overall response for the regulation of NUE traits in potatoes through information from the available sequence of potato genome (The Potato Genome Sequencing Consortium, 2011) (Tiwari et al., 2018).

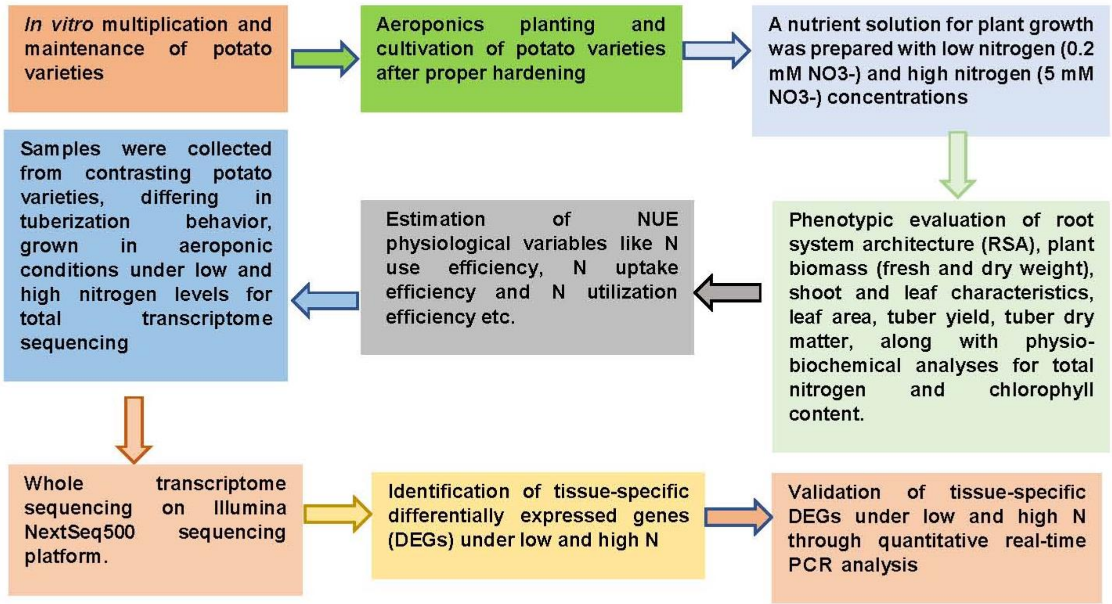

Fig 1.1. Illustration of the Work Plan Employed in the Present Study

Chapter-2
Review of Literature

CHAPTER 2 REVIEW OF LITERARTURE

2.1 Potato

Solanum tuberosum L., commonly known as potato and part of the *Solanaceae* family, is the world's third most important staple food crop after rice and wheat, playing a vital role in global food security. Additionally, it is the most important food crop that isn't a grain (Chakrabarti et al., 2017). Proteins, vitamins, minerals, and carbohydrates are all abundant in its tubers. They serve as a great source of energy and vital nutrients since they produce a lot of dry matter and calories per unit area and time. According to Koch et al. (2020), potatoes, starchy tubers that have been thickened for storing purposes, are one of the world's leading and widely grown food crops.

Potato plant is a herbaceous perennial, capable of surviving for multiple years without developing a woody stem. It typically grows to a height of 90–100 cm and is characterized by dark green leaves. During winter, the aerial parts senesce, with regrowth occurring in the spring and blooms after 3-4 weeks of its sprouting. It has white, pink, or purple flowers with yellow stamens. The potato's capability to form seeds after many years of its cultivation has been lost to an extent. There are very few potato flowers that bear fruit. Sometimes seeded to create new potato varieties, seed balls (berries) are tiny green tomatoes that resemble seeds and contain over 300 seeds each. Since they contain toxic substances, it is not advised to consume them.

While the above-ground parts die back during winter, the underground portion remains alive in dormant form and regenerates in the spring. Potato tubers are specialized stems connected to their root system, storing energy as starch and protein, along with water, to fuel future growth. Their outer skin periderm, protects them. The cortex is localized in this structure, which acts as a site for protein and starch storage. The starch is collected by vascular ring from the leaves and stem, which is situated inside the plant. Then, the starch enters the nearby parenchyma cells, which serve as a main starch storage site for tubers. A single plant usually produces 3 to 20 tubers during the growing season. In spring, the tubers begin to sprout, and the above-ground portion regrows.

The potato is believed to have originated in the Andes region, which includes present-day Peru and Bolivia, and was dispersed globally after Europeans encountered the Americas in the late 1500s. It is generally believed that first of all, the Portuguese brought the potato to India in the early 1600s, way before the adjoining countries. Later, the British introduced the crop to the Northern hills of India and Sri Lanka, where it became a fundamental crop in household gardens during the colonial period. By 1675, historical records indicate that the potato was already cultivated in places like Surat and Karnataka. Potato cultivation started in the Simla (now Shimla) hills in 1828, and by 1830, it had spread to the Nilgiri highlands. By the late 18th or early 19th century, the potato was a widely cultivated crop in the hills as well as the plains of India. Nevertheless, up until 1941, potato farming in India was limited, with the Indian subcontinent accounting for under 1% of the global area and production of potatoes (Singh, 2014).

Effective nutrient management is a key agronomic factor for successful potato production, in addition to variety selection, ongoing water delivery, and plant protection. An adequate amount of mineral nutrients is necessary, so that it

i) can protect the plant from adverse growth conditions,

ii) is required for maximum yield; and

iii) is crucial for producing high-quality potatoes (Koch et al., 2019).

The sixteen chemical elements that fit this description are carbon, hydrogen, oxygen, nitrogen, potassium, phosphorus, sulphur, calcium, magnesium, zinc, manganese, iron, copper, boron, molybdenum, and chloride. Thirteen out of these are derived from soil and fertilizers, while three—C, H, and O— are obtained from air and water. Legumes and other plants can use nitrogen that symbiotic organisms can extract from the atmosphere. The elements carbon, hydrogen, and oxygen are present in all organic materials. Carbon is a vital component of the carboxylic group, while nitrogen is the main element found in proteins, amino acids, and nucleic acids. Osmoregulation, glucose transport, and the activation of sonic enzyme systems all depend on potassium. The process of energy transfer is facilitated by phosphorus, which is available in phosphorylated sugars, alcohols, and lipids. Calcium, as a structural component of cell walls, contributes to membrane permeability, as well as cell division and elongation. The primary component of chlorophyll, and a crucial component of the phosphorylation reaction, is magnesium. Numerous amino acids contain sulphur. Zinc is a cofactor for tryptophan synthesis and other enzyme systems. In addition to being an indole-3-acetic acid (IAA) oxidase activator, manganese plays a part in photosynthetic activity. Electron transport, heme enzyme activity, and chlorophyll function all depend on iron. The activity of oxidase enzymes and the development of chloroplasts depend on copper. Cell wall development, cell differentiation, and glucose metabolism are all impacted by boron. The enzymes nitrogenase and nitrate reductase cannot function without molybdenum. As an osmoticum, chloride contributes to the activity of photosystem II (Westennann, 2005).

2.2 Nitrogen use efficiency (NUE)

Nitrogen is vital for the growth, yield, and quality of potato tubers. In contrast to other fertilizers, potatoes require more nitrogen fertilizer since they are a particularly nitrogen-intensive crop. For example, in northern India, farmers apply considerable amounts of nitrogen fertilizers, typically ranging from 180 to 280 kg per hectare, to reach tuber yields of 40 to 50 tonnes per hectare (Trehan and Singh, 2013). However, only 40 to 50 percent of the nitrogen applied is absorbed by plants, meaning that a large amount is lost to the environment (Ospina et al., 2014). Overuse of nitrogen fertilizers can result in difficulties including nitrate leaching and greenhouse gas emissions, which deteriorate soil, harm human health, and pollute the air and water. Therefore, in order to safeguard the environment, it is imperative to decrease the usage of nitrogen fertilizers and increase their effectiveness.

Enhancing plants' nitrogen usage efficiency (NUE) is a sustainable way to preserve agricultural output while safeguarding the environment. The ability of a plant to receive and use nitrogen for tuber production is measured by NUE. NUE for potatoes is calculated by dividing the tuber yield by the amount of nitrogen provided by fertilizer and soil. Although agronomic techniques and soil management have been used to maximize nitrogen utilization in potato crops, lowering the need for nitrogen fertilizers without sacrificing yield, there hasn't been much success in creating more efficient potato cultivars (Vos 1997; 2009). Even in nitrogen-limiting environments, genotypes with high NUE can provide yields comparable to high-yielding cultivars and react favorably to available nitrogen (Zebarth et al., 2004). Therefore, enhancing NUE in this significant crop requires an understanding of the genes linked to high-yielding, nitrogen-responsive potato cultivars (Fageria et al., 2008; Van Bueren et al., 2017).

Nitrogen (N) is considered the main macronutrient for the growth and development of biomass in plants. There are several methods in which a plant can use nitrogen. Their main sources are nitrate (NO_3^-) and ammonium (NH_4^+) (Silva et al., 2013), but nitrate is more likely to be leached. Nitrogen is a key component of chlorophyll, nucleic acids, coenzymes, amino acids, proteins, and membrane structures, all of which are crucial for plant development. (Andrews et al., 2013; Ahmed et al., 2015). The percentage of tubers that are considerable in size can be increased by increasing the amount of nitrogen available (Zebarth and Rosen, 2007). This is beneficial for potatoes intended for processing. Conversely, big tubers may not be ideal for consumption and seed production (Zebarth and Rosen, 2007). Additionally, tuber NO_3^- (Bélanger et al., 2002) and content of acrylamide (Gerendás et al., 2007) are two potato quality characteristics that are impacted by N. Nitrogen is the most influential factor affecting potato production out of the other major macronutrients (Bucher and Kossmann, 2011; Silva et al., 2013). N has the biggest impact on tuber weight. De la Morena et al. (1994) categorized potato yield mainly in three primary types:

i) the number of stems per square meter,

ii) the number of tubers per stem, and

iii) average tuber weight.

NUE can be defined as the tuber dry matter yield per unit of nitrogen supplied (Tiemens-Hulscher et al., 2014). Potatoes have a low NUE compared to other crops. This is because they have a shallow root system, which restricts their ability to absorb and use nitrogen (Iwama, 2008). Nonetheless, significant interactions between a particular cultivar's maturity rates and its NUE have been documented. According to Tiemens-Hulscher et al. (2014), late-maturing potato varieties are thought to benefit more from the enhanced nitrogen availability than their early-maturing counterparts. Moreover, the NUE may be significantly impacted by the water arrangement. In addition to their inefficient use of nutrients like nitrogen, potatoes are known for their shallow root structure, which makes them particularly vulnerable to scarcity of water (Liu et al., 2015). Therefore, even though too much water may encourage nitrogen leaching, in dry climates, potato crops generally require irrigation. As mentioned before, potatoes' shallow roots prevent them from absorbing nitrogen from the underlying soil depths (Cameron et al., 2013). As a result, the nitrate leaching possibility around the root zone is heightened which can increase further when there is sufficient water availability (Wolfe et al., 1983). Furthermore, both denitrification as nitrous oxide and volatilization as ammonia (Vos, 2009) are greenhouse gases that might result in adverse nitrogen loss (Petersen and Sommer, 2011). According to estimates that meet the real plant need, other nitrogen sources like catch crops and intercrops—that is, N-fixing leguminous plants—must be incorporated into an acceptable N source (Zebarth et al., 2012; Cameron et al., 2013; Bucher and Kossmann, 2011). On potatoes, 100–300 kg of nitrogen is usually sprayed per hectare (Beukema and Van Der Zaag 1990).

2.3 Transcriptome analysis

There is little information available on the genes involved in potatoes' nitrogen (N) metabolism. Therefore, it is imperative to collect additional data on gene expression profiles in N-responsive genotypes in a variety of situations, including controlled and field settings (Gálvez et al., 2016). Numerous important genes, including those involved in absorption, translocation, assimilation/utilization, and remobilization, have been discovered in prior research as being involved in the nitrogen metabolism process. Nitrate transporters, ammonium transporters, nitrate reductase, nitrite reductase, glutamine synthetase, and asparagine synthetase are important genes in this process. The sequencing of the potato genome in 2011 (Potato Genome Sequencing Consortium, 2011) has led to a significant increase in the availability of transcriptomic data in the literature. Genes linked to potato nitrogen metabolism have been identified by a few transcriptome studies. Similar gene networks have also been found in potatoes by multi-omics analysis of the overuse of nitrogen fertilizer. So far, most research has been undertaken in field settings, identifying genes that are likely implicated in nitrogen use efficiency (NUE) in potatoes (Tiwari et al., 2020; Guo et al., 2022).

Nitrogen is considered a key source of minerals for plant development, out of which the bulk is carried by nitrate transporters (*NRTs*). Zhang et al. (2021) discovered members of the gene family of *StNRT* in potatoes and classified the *StNRT* subfamily, identified its gene structure, and analyzed its arrangement, in addition to predicting its conserved domain using a variety of bioinformatic tools. It was determined that separate members are found in various tissues, particularly in the presence of elevated nitrogen levels. These results proved beneficial in identifying components of the *StNRT* family in potatoes and may help in future research on the functional characterisation of *StNRT* genes (Zhang et al., 2021).

Zhang and co-workers in 2020 found the function of glutamine synthetase (*GS*) and nitrate reductase (*NR*) in potato. Most differentially expressed genes (DEGs) in different N treatments are involved in N metabolism and nitrogen molecule transport, exhibiting a genotype-dependent reaction to nitrogen shortage. DEGs like carbonic anhydrase (*StCA*), glutamine synthetase (*StGS*), and glutamate dehydrogenase (*StGDH*) are crucial for the aforementioned processes. DEGs related to N metabolism showed a strong link with nitrogen utilization efficiency (NUtE), but not with nitrogen use efficiency (NUE). It has been demonstrated that nitrate transporter 2.4 (*StNRT2.4*), 2.5 (*StNRT2.5*), and 2.7 (*StNRT2.7*), members of the Major Facilitator Superfamily (MFS), are enriched in defense and stress response pathways.

A diverse response was observed by Tiwari et al. (2022) in varieties for root system architecture (RSA), plant biomass, NUE, and yield contributing traits under optimum N conditions. It was true regardless of when potato varieties were released from the 1960s to 2018 and whether the varieties' unique characteristics suggested that different parents were used in the breeding process. Kufri Lalit, Kufri Frysona, Kufri Kumar, Kufri Alankar, Kufri Neela, Kufri Pushkar, Kufri Khyati, and Kufri Arun were found to be high-yielding varieties (> 150 g) under aeroponic conditions, while Kufri Ashoka, Kufri Badshah, Kufri Mohan, Kufri Sutlej, Kufri Chipsona-3, Kufri Garima, Kufri Giriraj, Kufri Bahar, Kufri Jyoti, Kufri Neelkanth, Kufri Kesar, Kufri Jawahar, and Kufri Kundan were medium-yielding (100-150 g). Overall, popular and high-yielding cultivars performed superior in terms of plant biomass, RSA, tuber yield, AgNUE and NUE.

The importance of understanding the anatomy, function and root architecture of a particular crop for nutrient-efficient crop breeding. In potatoes, it is yet unknown how the basal and stolon root architecture relates to the tuber yield and vis-à-vis carbon partitioning. The advancement of modern technologies such as sensors, robotics, cameras, and High-Throughput Phenotyping (HTP) platforms has enabled the analysis of root architecture and phenomics-based crop breeding. Therefore, using contemporary genomics technologies to create abiotic stress-tolerant cultivars requires a fresh focus on root architecture.

Shrestha et al. (2023) found that, depending on factors such as climate, variety, soil type, and water availability, applying light-to-moderate deficit irrigation (10–30% of full irrigation) combined with reduced nitrogen rates (60–170 kg/ha) can significantly enhance water and nitrogen use efficiencies, while still achieving maximised yield and quality in potato production. By reducing nitrate leakage outside of the crop root zone, deficit irrigation techniques can lower N application rates in potato production. Furthermore, less NO_3^- nitrogen is leached to deeper soil layers as a result of soil N mobility and prudent N treatment scheduling, especially when done every week. This study's framework for N scheduling aimed to match the plant growth curve can maintain the best crop yield while decreasing NO_3^- nitrogen leaching and boosting N absorption and recovery (Badr et al., 2023).

An outline of essential genes involved in tuberization under high temperature stress in the potato variety Kufri Anand, grown in aeroponics, is provided through the identification of several key genes associated with tuberization under heat stress. These include heat shock proteins (such as the 18.5 kDa class I heat shock protein), sugar metabolism genes (like glucosyltransferase), transcription factors (such as WRKY), and phytohormones (e.g., auxin-induced beta-glucosidase) (Zinta et al., 2024a). Thus, the work cleared the way for the identification of possible genes linked to potato tuber yield characteristics grown in an aeroponic system.

2.4 Aeroponic system

According to Buckseth et al. (2016), the aeroponic system is a soilless growing technique that uses liquid or mist formulations to supply nutritional solutions straight to the plant root zone under dark conditions. This process works very well for the production of potato minitubers of superior quality. Sharifi et al. in 2007 screened potato genotypes using hydroponics and *in vitro* culture (Schum and Jansen, 2014). The aeroponic system has the potential to revolutionize potato production because of its many benefits, including rapid seed production, root system architecture, and growth, as well as good nutrition monitoring. According to Buckseth et al. (2016), the precision phenotyping of potatoes has previously been established using aeroponic technology, and it has enormous promise for further research. Utilizing germplasm/variety potential to increase NUE in potatoes has recently drawn attention (Ospina et al., 2014; Van Bueren and Struik, 2017), especially concerning root phenotyping (Tracy et al., 2020).

'Aeroponics' is a technology that has greatly changed the potato industry in recent decades. This method was developed to utilize healthy *in vitro* plants for the production of minitubers (Buckseth et al., 2016), which makes it possible to grow potatoes all year while adhering to phytosanitary regulations. In the aeroponic system, microplants are placed at the brink of a growth chamber, while on the inside, the root part is misted with a nutrient-enriched solution (Tierno et al., 2014; and Buckseth et al., 2016). An insect-proof net home is there in the growth chambers. To accommodate the potato plants, the aeroponic unit has a detachable top, containing holes. Because of the pivots on the front of the chamber, minitubers of the appropriate size can be harvested at various intervals. Harvesting commences 40 to 50 days after planting, depending upon genotype, once the tubers reach a size of 3 to 10 grams. Minitubers are collected weekly, resulting in approximately 10 to 12 harvests throughout the four to five-month crop season (Tiwari et al., 2019). Depending on the variety, one *in vitro* plant can generally produce 40–50 minitubers, significantly more than the 8–10 minitubers typically obtained conventionally from net-house nursery beds (Buckseth et al., 2020). For planting in the next crop season, the harvested minitubers are kept in storage at temperatures ranging from 2-4°C. Although it requires substantial planning, operational investment, and tailored nutritional solutions for different genotypes, this system has revolutionized seed potato production in India by generating high-quality seed tubers (Buckseth et al., 2022). It has also been shown that Indian varieties grown in aeroponic settings differ in terms of root morphology and yield traits (Tiwari et al., 2022).

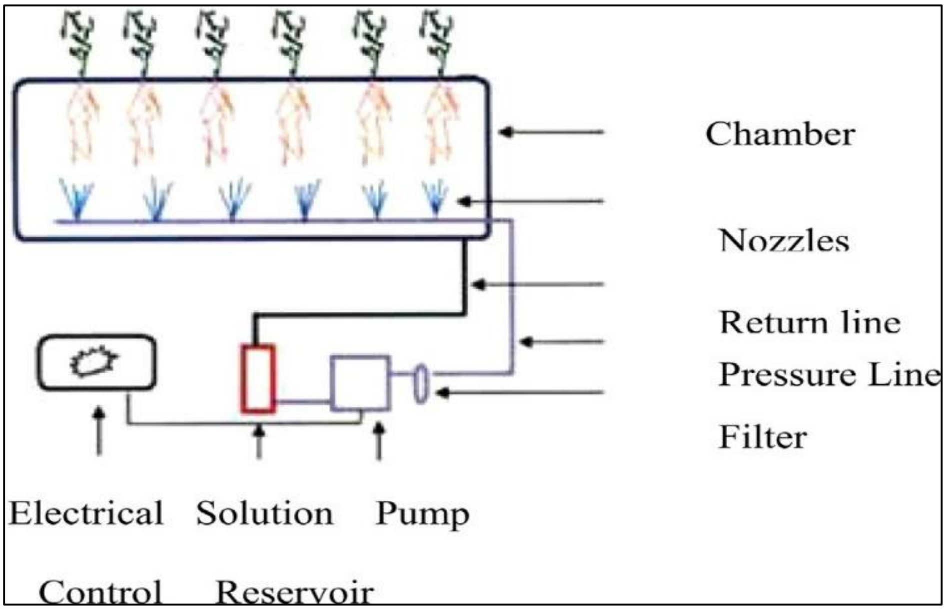

Fig 2.1. Layout of the Aeroponic system for potato cultivation

Fig 2.2. Full crop growth stage of Indian potato varieties grown at the Aeroponic system installed at ICAR-CPRI, Shimla.

2.5 Research Gaps

1. In potatoes, numerous studies on the management of soil-agronomic nitrogen and a few on the analysis of genetic diversity have been carried out in India (Trehan et al., 2008; Trehan 2009; Singh and Trehan, 2013). However, there is a lack of reports on NUE research related to aeroponics and genomics approaches at the plant genetic level to improve NUE. At ICAR-CPRI, the physiology, integrated genomics, and breeding approaches for improving NUE in potatoes have been conceptualized (Tiwari et al., 2018*).* The other studies highlighted the application of aeroponics in precision phenotyping to investigate potato root system biology (Tiwari et al., 2019) and also seed potato production (Buckseth et al., 2016). Recently, we have worked on the identification of homologous candidate genes and also analyzed transcriptomes to identify genes and microRNAs responsible for N metabolism to improve NUE in potatoes.
2. The perception of plant N control has expanded in model plants and cereals with the introduction of genomics (Kraiser et al., 2011), but it is still relatively unknown in potatoes. Despite efforts by many plant scientists to identify or develop nitrogen-use-efficient genotypes, the release of such varieties has been limited, largely due to the complexity of root genetics. In potatoes, the mechanisms and genetic factors underlying traits related to NUE—such as root

architecture, carbon-nitrogen balance, nitrogen uptake, and utilization—are still not fully understood. Thus, understanding key regulatory genes, traits, and phenotypes is crucial for identifying tuberization-related genes under varying nitrogen conditions and examining root system architecture to improve nitrogen use efficiency in Indian potato cultivars.

3. To improve NUE in potatoes, strategies based on genomics, integrated breeding, and physiology have been proposed, drawing inspiration from other plants like rice, maize, *Arabidopsis thaliana*, and wheat (Tiwari et al., 2018). They have also effectively conducted phenotyping of potatoes grown in aeroponic systems by using carefully controlled macro- and micronutrient levels, including low nitrogen doses, without compromising tuber yield. (Tiwari et al., 2019). With the accessibility of the potato genome sequence and the rise of cost-effective sequencing technologies, it is now feasible to identify the genes affecting N metabolism in potatoes. To the best of our knowledge, using RNA-seq techniques, Gálvez et al. (2016) identified nitrogen-responsive genes and regulatory motifs in potatoes grown in the field.

This study aimed to identify genes and regulatory factors linked to nitrogen deficiency (low N) and sufficiency (high N, control) in Indian potato varieties cultivated in aeroponic culture under controlled conditions, using RNA-seq-based transcriptome analysis. The proposed research is also directed to standardize nitrogen uptake across different potato varieties and reduce nitrogen bioleaching into the environment. It will help identify key regulatory genes and traits crucial for dissecting phenotype, understanding tuberization under various nitrogen conditions, and analyzing root system architecture to improve nitrogen use efficiency in Indian potato cultivars. Identifying the genes responsible for tuberization in several potato cultivars by applying varying nitrogen supplies was another goal. This will improve our knowledge of how to change the genes involved in N metabolism for future genetic manipulation. Apart from our research, very little has been published to date on the genes linked to N metabolism in potatoes that are subjected to field circumstances rather than precision phenotyping in aeroponics. Hence, to better understand the tuberization behavior of several potato varieties grown in a soilless aeroponic environment, this study will identify the genes, agro-physio-biochemical, and root architecture features linked to NUE under varying N regimes.

Chapter-3
Hypothesis

CHAPTER 3 HYPOTHESIS

Potato is a tuber crop that has a significant role in providing global food and nutritional security. Due to the short and shallow root structure, potatoes have a low N-recovery and nitrogen utilization efficiency (NUE), which must be overcome by a high N need for vegetative development and output. This results in pollution through nitrate leaching and greenhouse gas emissions (N oxides), which contaminate water supplies and degrade soil fertility and air quality. Therefore, one possible strategy to deal with these issues is to examine the traits that improve a plant's NUE. Additionally, for the development of high-quality seed potatoes (mini tubers), as well as for genomic, physiologic, and breeding purposes in potatoes, as well as for displaying transcriptome analysis under N stress, aeroponic technology—a soilless and mist-based fertilizer administration system—has been utilized globally. Precision phenotyping of potatoes has been shown using aeroponic technology, which also holds great promise for increasing NUE in potato germplasm/variety through root phenotyping. Therefore, we hypothesize that RNA-sequencing-based transcriptome analysis can be used to identify genes and regulatory elements related to nitrogen deficiency (low N) versus adequate nitrogen (high N, control) in Indian potato varieties grown under controlled conditions of aeroponic culture. The proposed research aims to standardize nitrogen uptake across different varieties and reduce nitrogen bioleaching into the environment. It will provide insights into the role of key regulatory genes and traits essential for understanding phenotype, identifying tuberization-related genes under varying nitrogen conditions, and analyzing root system architecture to improve nitrogen use efficiency in Indian potato cultivars. The genes taking part in the tuberization process in various potato types under varying N supplies will also be identified in this investigation. This will improve our knowledge of how to change the genes involved in N metabolism for future genetic manipulation. Therefore, this study will identify the genes, agro-physio-biochemical, and root architecture characteristics that contribute to NUE in various potato varieties and their tuberization behavior under varying N circumstances, and thereby can pave the way to develop N-use efficient cultivars through integrated genomics, physiology and breeding methods.

Chapter-4
Objectives

CHAPTER 4 OBJECTIVES

1. To evaluate agronomical and physio-biochemical traits in potato varieties under differentnitrogen regimes in aeroponics.
2. To analyze transcriptome dynamics in potato for tuberization under different N conditions in aeroponics.
3. To dissect root system architecture in potato varieties under varied nitrogen supply inaeroponics.

Chapter-5
Materials and Methods

CHAPTER 5 MATERIALS AND METHODS

5.1 Plant material

Fifty-six Indian potato varieties, released during 1960-2020, were used from the Germplasm Unit of the Division of Crop Improvement and Seed Technology, Indian Council of Agricultural Research-Central Potato Research Institute (ICAR-CPRI), Shimla, Himachal Pradesh, India (31.1048° N, 77.1734° E, 2,276 m above sea level). The experiment was carried out in a Completely Randomized Design (CRD) under controlled aeroponic conditions. Potato varieties were first multiplied in abundant quantity under *in vitro* (tissue culture) conditions on a hormone-free MS medium for planting under aeroponics.

Table 5.1 Detail of potato varieties used in this study

S. No.	Name of the Variety	Year of its release	Special traits
1	Kufri Alankar	1968	Shows moderate resistance to late blight and early bulker.
2	Kufri Anand	1999	Shows moderate resistance to late blight, is tolerant to hopper burn and frost, and is suitable for the spring season.
3	Kufri Ashoka	1996	Susceptible to late blight.
4	Kufri Badshah	1979	Resistant to late blight, early blight and PVX.
5	Kufri Bahar	1980	Susceptible to late blight, and shows moderate resistance to gemini virus and early bulker.
6	Kufri Chamatkar	1968	Susceptible to late blight and mainly forms medium-sized tubers.
7	Kufri Chandramukhi	1968	Susceptible to late blight and forms attractive tubers with excellent flavour.
8	Kufri Chipsona-1	1998	Shows resistance to late blight and is suitable for chips and French fries.
9	Kufri Chipsona-3	2006	Shows resistance to late blight and is suitable for chips & French fries.
10	Kufri Chipsona-4	2010	Suitable for chips.
11	Kufri Dewa	1973	Frost-tolerant and a good keeper.
12	Kufri FryoM	2019	Shows resistance to late blight and PVY, and is suitable for French fries.
13	Kufri Frysona	2009	Shows resistance to late blight and is suitable for

S. No.	Name of the Variety	Year of its release	Special traits
			French fries.
14	Kufri Ganga	2018	Shows moderate resistance to late blight and is tolerant to moderate drought conditions.
15	Kufri Garima	2012	Resistant to late blight.
16	Kufri Gaurav	2012	Susceptible to late blight and is nutrient (NPK) use efficient at sub-optimal doses.
17	Kufri Girdhari	2008	Shows high resistance to late blight and has a long tuber dormancy.
18	Kufri Giriraj	1998	Moderately resistant to late blight.
19	Kufri Himalini	2006	Moderately resistant to late blight, and has a good yield in both hills & plains.
20	Kufri Himsona	2008	Moderately resistant to late blight and is suitable for chips.
21	Kufri Jawahar	1996	Shows moderate resistance to late blight, has slow degeneration and is suitable for intercropping.
22	Kufri Jeevan	1968	Shows moderate resistance to late and early blight.
23	Kufri Jyoti	1968	Shows moderate resistance to late blight, early bulker, has a wide adaptability, slow degeneration and is day neutral.
24	Kufri Kanchan	1999	Shows moderate resistance to late blight and has slow degeneration.
25	Kufri Karan	2019	Highly resistant to late blight, six potato viruses and potato cyst nematodes.
26	Kufri Khasigaro	1968	Shows moderate resistance to late blight and early blight.
27	Kufri Kesar	2017	Susceptible to late blight.
28	Kufri Khyati	2008	Shows resistance to late and early blight, early bulker and is suitable for high cropping intensity.
29	Kufri Kuber	1958	Susceptible to late blight.
30	Kufri Kumar	1958	Shows moderate resistance to late blight.
31	Kufri Kundan	1958	Shows moderate resistance to late blight.
32	Kufri Lalima	1982	Shows moderate resistance to late blight.
33	Kufri Lalit	2014	Shows resistance to late blight.
34	Kufri Lauvkar	1972	Susceptible to late blight and heat tolerance.
35	Kufri Lima	2018	Susceptible to late blight, extremely resistant to PVX and PVY, tolerant to early heat, hopper burn

S. No.	Name of the Variety	Year of its release	Special traits
			and mite, and suitable for early and main planting.
36	Kufri Manik	2019	Shows resistance to late blight and is rich in micronutrients (Fe and Zn), anthocyanins and carotenoids, and is suitable for eastern plains (bio-fortified variety).
37	Kufri Megha	1989	Shows moderate resistance to late blight.
38	Kufri Mohan	2016	Shows moderate resistance to late blight.
39	Kufri Muthu	1971	Shows moderate resistance to late blight.
40	Kufri Naveen	1968	Shows moderate resistance to late blight.
41	Kufri Neela	1963	Moderately resistant to late blight.
42	Kufri Neelkanth	2018	Shows resistance to late blight, is rich in antioxidants (anthocyanins and carotenoids), has excellent flavour and is a specialty potato.
43	Kufri Pukhraj	1998	Shows moderate resistance to late blight, early bulker and requires low input.
44	Kufri Pushkar	2005	Shows resistance to late blight.
45	Kufri Red	1958	Susceptible to late blight.
46	Kufri Sadabahar	2008	Shows moderate resistance to late blight and early bulker.
47	Kufri Sahyadri	2019	Highly resistant to potato cyst nematodes & shows moderate resistance to late blight.
48	Kufri Sangam	2019	Shows moderate resistance to late blight and has excellent storability.
49	Kufri Sutlej	1996	Shows moderate resistance to late blight.
50	Kufri Sherpa	1983	Shows resistance to late blight.
51	Kufri Sindhuri	1967	Susceptible to late blight and is suitable for low-input area.
52	Kufri Sukhyati	2017	Moderately resistant to late blight.
53	Kufri Surya	2006	Susceptible to late blight, has heat tolerance and hopper burn resistance, and is suitable for early planting.
54	Kufri Swarna	1985	Shows resistance to late blight and PCN.
55	Kufri Thar-1	2019	Shows drought tolerance (20% water saving), and is suitable for Orissa & UP.
56	Kufri Thar-2	2019	Shows drought tolerance (20% water saving), and is suitable for UP, Rajasthan, Haryana, and

S. No.	Name of the Variety	Year of its release	Special traits
			Chhattisgarh.

5.2 *In vitro* regeneration of plants

5.2.1 Murashige and Skoog (MS) medium composition

Stock solution

MS Stock- 1-Nitrates

Name of Chemical	Strength (× 50)	100 ml	250 ml	500 ml	1000 ml
NH_4NO_3 1650 mg/L	20 ml for 1L medium	8.250 g	20.625 g	41.250 g	82.500 g
KNO_3 1900 mg/L		9.500 g	23.750 g	47.500 g	95.000 g

MS Stock- 2-Sulphates

Name of Chemical	Strength (× 100)	100 ml	250 ml	500 ml	1000 ml
$MgSO_4.7H_2O$ 370 mg/L	10 ml for 1L medium	3.700 g	9.250 g	18.500 g	37.000 g
$MnSO_4.H_2O$ 16.9 mg/L		169 mg	423 mg	845 mg	1690 mg
$ZnSO_4.7H_2O$ 8.6 mg/L		86 mg	215 mg	430 mg	860 mg
$CuSO_4.5H_2O$ 0.025 mg/L		0.25 mg (1.0 ml)	0.625 mg (2.5 ml)	1.25 mg (5.0 ml)	2.5 mg (10.0 ml)
Dissolve 25 mg $CuSO_4.5H_2O$ in 100 ml dH_2O and then add the required volume to the MS 2 Stock					

MS Stock- 3

Chemical	Strength (× 100)	100 ml	250 ml	500 ml	1000 ml
$CaCl_2.2H_2O$ 440 mg/l	10 ml for 1L medium	4.400 g	11.000 g	22.000 g	44.000 g
KI		8.3 mg	21.0 mg	41.5 mg	83.0 mg

0.83 mg/L					
$CoCl_2.6H_2O$ 0.025 mg/L		0.25 mg (1.0 ml)	0.625 mg (2.5 ml)	1.25 mg (5.0 ml)	2.5 mg (10.0 ml)
Dissolve 25mg $CoCl_2.6H_2O$ in 100 ml dH_2O and then add the required volume to MS 3 Stock					

MS Stock- 4

Chemical	Strength (× 100)	100 ml	250 ml	500 ml	1000 ml
KH_2PO_4 170 mg/L	10 ml for 1L medium	1.700 g	4.250 g	8.500 g	17.000 g
H_3BO_3 6.2 mg/L		62.0 mg	155 mg	310 mg	620 mg
$NaMoO_4.2H_2O$ 0.25 mg/L		2.5 mg (1.0 ml)	6.25 mg (2.5 ml)	12.5 mg (5.0 ml)	25.0 mg (10.0 ml)
Dissolve 250mg $NaMoO_4.2H_2O$ in 100 ml dH_2O and then add the required to MS 4 Stock					

MS Stock- 5

Chemical	Strength (× 100)	100 ml	250 ml	500 ml	1000 ml
$FeSO_4.7H_2O$ 27.8 mg/L	10 ml for 1L medium	278 mg	695 mg	1390 mg	2780 mg
$Na_2EDTA.2H_2O$ 37.3 mg/L		373 mg	933 mg	1865 mg	3730 mg
Store in amber colour bottle					

MS Stock- 6-Vitamins

Chemical	Strength (× 1000)	100 ml
Thiamine-HCl (0.1 mg/L)	1 ml for 1L medium	10.0 mg
Pyridoxine-HCl (0.5 mg/L)		50.0 mg
Nicotinic acid (0.5 mg/L)		50.0 mg

Glycine (2.0 mg/L)		200.0 mg
Store at 0°C		

To be added directly

- ➢ Myo-Inositol: 100 mg/L
- ➢ Sucrose: 20 g/L
- ➢ pH: 5.8
- ➢ Gelrite: 2 g/L
- ➢ Autoclave-sterilize: 121 °C for 20 minutes.

5.3 Aeroponic cultivation of potato varieties

- ∗ Potato varieties: 56
- ∗ Nitrogen treatments: 2 (High N: 5 mM; and Low N: 0.5 mM)
- ∗ Replications: 2
- ∗ Design: Completely Randomized Design (CRD)
- ∗ Growth conditions: Aeroponics (ICAR-CPRI, Shimla, Himachal Pradesh, India)
- ∗ Using our conventional tissue culture techniques, 56 different potato cultivars were multiplied *in vitro* for the aeroponics experiment. Evaluation of genotypes in aeroponics with contrasting nitrogen regimes, i.e., high N (5 mM) and low N (0.5 mM), (10 plants of each potato genotype and completely randomized design with 2 replications grown over the period of two years, 2021-22 and 2022-23). With 11 hours of light and 13 hours of darkness, the plants were cultivated in a controlled setting with a daily temperature of 23 ± 2°C.
- ∗ The nutrient solution was prepared for aeroponic plant growth with two supplies; low N (0.5 mM N) and high N (5 mM N). Several salts were used in the high N (5 mM) treatment, including NH_4NO_3 (0.5 mM), $Ca(NO_3)2.4H2O$ (1 mM), KNO_3 (2 mM), KH_2PO_4 (0.5 mM), $MgSO_4.7H2O$ (1 mM), NaCl (0.125 mM), Fe-EDTA (0.0062 mM), H_3BO_3 (0.004 mM), $MnSO_4.H_2O$ (0.0016 mM),

$ZnSO_4.7H_2O$ (0.00008 mM), $CuSO_4.5H_2O$ (0.00004 mM), $Na_2MoO_4.2H_2O$ (0.00004 mM), $CoCl_2.6H_2O$ (0.00004 mM).

* The low nitrogen treatment (0.5 mM) included NH_4NO_3 (0.25 mM), KH_2PO_4 (0.5 mM), K_2SO_4 (1 mM), $CaSO_4.2H_2O$ (1 mM), $MgSO_4.7H_2O$ (1 mM), NaCl (0.125 mM), Fe-EDTA (0.0062 mM), H_3BO_3 (0.004 mM), $MnSO_4.H_2O$ (0.0016 mM), $ZnSO_4.7H_2O$ (0.00008 mM), $CuSO_4.5H_2O$ (0.00004 mM), $Na_2MoO_4.2H_2O$ (0.00004 mM), $CoCl_2.6H_2O$ (0.00004 mM).

* To cultivate a robust potato crop, nutrient solutions were switched out every seven days and pH was maintained between 5.8–7.0 using either H_2SO_4 or NaOH. The growth chamber of the aeroponic system was kept at or less than 18–20°C at night and 23–25°C during the day, along with an average light intensity of about 200±10 µM m-2s-1. Shorter days encourage the tuberization process of potatoes.

* After sowing, the ultimate crop was harvested 110 days later. At least three plants in each replication had their traits documented. At 60 DAP days following planting, samples of leaves and tubers were taken from both treatments. 110 days after planting (DAP), the crop was harvested after completing its life cycle. Three replications of each N treatment were used, and at least three plants in each replication had all the features noted.

5.4 Composition of aeroponic solutions

Table 5.2: 5 N Mix High N

S. No.	Chemical	MW	Stock conc. (M)	Salt weight (g/L) stock	Stock vol. (ml) for 1 L working sol.	Final conc. (mM)	Vol (ml) for 250 L	Nutrient	mM
1	NH_4NO_3	80.04	1.0	80.04	0.5	0.5	125	N (NO_3 & NH_4)	5.00
2	KH_2PO_4	136.09	0.5	68.045	1.00	0.5	250	P	0.5
3	K_2SO_4	174.26	1.0	174.26				K	2.5
4	$CaSO_4.2H_2O$	172.17	0.5	86.085				Ca	1.00
5	$MgSO_4.7H_2O$	246.47	1.0	246.47	1.00	1.0	250	Mg	1
6	NaCl	58.44	0.5	29.22	0.25	0.125	62.5	S	1.00172
7	Fe-EDTA	367.1	0.0125	4.5888	0.50	0.0062	124	Na	0.1251
8	H_3BO_3	61.83	0.125	7.7288	0.03	0.004	8	Fe	0.0062
9	$MnSO_4.H_2O$	169.02	0.125	21.1275	0.01	0.0016	3	Mn	0.0016
10	$ZnSO_4.7H_2O$	287.54	0.0125	3.5943	0.01	0.00008	2	Zn	0.00008
11	$CuSO_4.5H_2O$	249.68	0.005	1.2484	0.01	0.00004	2	B	0.004
12	$Na_2MoO_4.2H_2O$	241.95	0.005	1.2098	0.01	0.00004	2	Cu	0.00004
13	$CoCl_2.6H_2O$	237.93	0.005	1.1897	0.01	0.00004	2	Mo	0.00004
14	$(NH_4)_2SO_4$	132.14	0.5	66.07				Cl	0.1251
15	Ca (NO_3)2.4H_2O	236.15	1.0	236.15	1.00	1.0000	250	Co	0.00004
16	KNO_3	101.1	1.0	101.1	2.00	2.0000	500		

Table 5.3: 0.5N Mix Low N

S. No.	Chemical	MW	Stock conc. (M)	Salt weight (g/L) stock	Stock vol. (ml) for 1 L working sol.	Final conc. (mM)	Vol (ml) for 250 L	Nutrient	mM
1	NH_4NO_3	80.04	1.0	80.04	0.25	0.25	62.5	N (NO_3 & NH_4)	0.50
2	KH_2PO_4	136.09	0.5	68.045	1.00	0.5	250	P	0.5
3	K_2SO_4	174.26	1.0	174.26	1.00	1.0000	250	K	2.5
4	$CaSO_4.2H_2O$	172.17	0.5	86.085	2.00	1.0000	500	Ca	1.00
5	$MgSO_4.7H_2O$	246.47	1.0	246.47	1.00	1.0	250	Mg	1
6	NaCl	58.44	0.5	29.22	0.25	0.125	62.5	S	1.00172
7	Fe-EDTA	367.1	0.0125	4.5888	0.50	0.0062	124	Na	0.1251
8	H_3BO_3	61.83	0.125	7.7288	0.03	0.004	8	Fe	0.0062
9	$MnSO_4.H_2O$	169.02	0.125	21.1275	0.01	0.0016	3.2	Mn	0.0016
10	$ZnSO_4.7H_2O$	287.54	0.0125	3.5943	0.01	0.00008	1.6	Zn	0.00008
11	$CuSO_4.5H_2O$	249.68	0.005	1.2484	0.01	0.00004	2	B	0.004
12	$Na_2MoO_4.2H_2O$	241.95	0.005	1.2098	0.01	0.00004	2	Cu	0.00004
13	$CoCl_2.6H_2O$	237.93	0.005	1.1897	0.01	0.00004	2	Mo	0.00004
14	$(NH_4)_2SO_4$	132.14	0.5	66.07	0.00	-	-	Cl	0.1251
15	Ca $(NO_3)2.4H_2O$	236.15	1.0	236.15	0.00	-	-	Co	0.00004
16	KNO_3	101.1	1.0	101.1	0.00	-	-		

Table 5.4: Comparison

Nutrient	**5 N High N (mM)**	**0.5 N Low N (mM)**
N as $NO_3^-NH_4$	5	0.5
P	0.5	0.5
K	2.5	2.5
Ca	1	1
Mg	1	1
S	1.00172	3.00172
Na	0.12508	0.12508
Fe	0.0062	0.0062
Mn	0.0016	0.0016
Zn	0.00008	0.00008
B	0.004	0.004
Cu	0.00004	0.00004
Mo	0.00004	0.00004
Cl	0.12508	0.12508
Co	0.00004	0.00004

5.5 Observations recorded

Following observations were recoded in all 56 potato varieties grown in two N regimes (high N and low N) in three replications under aeroponics.

5.5.1 Plant height (cm)

At 60 days after planting, when plants reached good vegetative growth, plant height (cm) was measured from a minimum of three plants per replication on a per-plant basis in 56 different potato varieties.

5.5.2 Total leaf area (cm^2)

The LI-3100C Area Meter (LICOR Biosciences, Lincoln, Nebraska, USA) was used to measure the total leaf area per plant after all of the leaves had been removed. At 60 days after planting, the total leaf area (cm^2) of 56 different potato varieties was measured from at least three plants per replication on a per plant basis.

5.5.3 Total chlorophyll (mg / g FW)

At 60 days following planting, the leaves (more precisely, the fourth leaf from the top) of both low-nitrogen (N) and high-nitrogen (N) fed plants were examined to assess the total chlorophyll content (in milligrams per gram of fresh weight).

Total chlorophyll content was estimated using the protocols described by Anderson & Boardman (1964).

- 100 mg of fresh sample in the form of leaves was crushed finely in 3 ml of 80% acetone and collected in a tube.
- The tube was centrifuged at 5000 rpm for ten minutes, the supernatant was transferred to another fresh tube (a) while the remaining pellet was suspended in 3ml of 80% acetone and vortexed thoroughly.
- The suspension was again centrifuged at 5000 rpm for ten minutes, and supernatant was transferred to the previous fresh tube (a) and volume was made upto 10 ml with 80% acetone.
- A UV-1700 Spectrophotometer from Shimadzu Corporation (Kyoto, Japan) was used to measure the absorbance of the chlorophyll at wavelengths of 645 nm and 663 nm, with 80% acetone serving as a blank.
- The total chlorophyll content was estimated using the following formulas:

$$\text{Total Chl} \quad = \quad 20.2\,(OD_{645}) + 8.02\,(OD_{663}) \times \frac{V}{1000 \times W}$$

Where, OD_{663} = OD at 663 nm

OD_{645} = OD at 645 nm

V = Total volume of supernatant (ml)

W = Weight of sample (g)

Total chlorophyll content was expressed as mg/g fresh weight.

5.5.4 Root dry weight (g)

At 110 DAP, the crop was harvested. At the harvest stage, shoot dry weight (g) was calculated for each plant. The roots were dried in an oven (Binder, Tuttlingen, Germany) at 70℃ for four to five days until their constant weight was achieved. An electronic balance (Mettler Toledo, Ohio, USA) was then used to measure the dry weight. For each replication, data were collected from a minimum of three plants per replication.

5.5.5 Shoot dry weight (g)

Similar to above, shoot dry weight was calculated on a per-plant basis at the harvest stage (110 days after planting). The shoots were dried in a hot air oven (Binder, Tuttlingen, Germany) at 70℃ for four to five days until their weight was constant. An electronic balance (Mettler Toledo, Ohio, USA) was then used to measure the dry weight. For each replication, data were collected from a minimum of three plants per replication.

5.5.6 Tuber dry matter (%)

As above, tuber dry matter (%) was estimated on a per plant basis at the harvest stage (110 days after planting). The tubers were dried in an oven (Binder, Tuttlingen, Germany) at 70ºC for four to five days until their constant weight was achieved. An electronic balance (Mettler Toledo, Ohio, USA) was then used to measure the dry weight. On a per-plant basis, data were collected from a minimum of three plants in every replication.

5.5.7 Tuber number/plant

Tuber harvesting was done from all the plants in 56 potato varieties upto the harvest stage (110 days after planting, DAP) on per per-plant basis. Harvesting started from 45 DAP and continued up to 110 DAP.

- $\text{Tuber number/plant} = \frac{\text{Total number of tuber harvested}}{\text{Total number of plants}}$

5.5.8 Tuber yield /plant (g)

As above, tuber harvesting was done from all the plants in 56 potato varieties upto the harvest stage (110 days after planting, DAP) on per plant basis. Harvesting started from 45 DAP and continued upto 110 DAP. Total tuber yield was estimated from the sum of all the harvests.

- Tuber yield /plant (g) $= \frac{\text{Tuber yield (g)}}{\text{Total number of plants}}$

5.5.9 Root length (cm)

Root length was measured per plant for each of the 56 potato varieties at 60 DAP, using at least three plants in three replications for Root System Architecture (RSA) profiling. The 'EPSON Expression 12000XL' root scanner (Seiko Epson Corporation, Suwa-shi, Nagano-ken, Japan) was used to measure the root length, and WinRHIZO Pro 2020a software was used to analyze the scanned pictures. (Regent Instruments Inc., Quebec, Canada 2020a) (Arsenault et al., 1995; Regent Instrument Inc., Quebec, Canada). Using the WinRHIZO Pro 2020a software's default settings, various root classes (ranging from 0 to < 0.5, 0.5 to < 1, 1 to < 1.5, 1.5 to < 2, 2 to < 2.5, 2.5 to < 3, 3 to < 3.5, 3.5 to < 4, 4 to < 4.5, and > 4.5 mm) were analysed for total root length, total surface area, and volume.

5.5.10 Root surface area (cm^2)

At 60 DAP, the root surface area of at least three plants in three replications was measured on a per-plant basis. The 'EPSON Expression 12000XL' root scanner (Seiko Epson Corporation, Suwa-shi, Nagano-ken, Japan) was used to measure the root surface area. The 'WinRHIZO Pro 2020a' software (Regent Instruments Inc., Quebec, Canada) was used to analyze the scanned images of roots.

5.5.11 Root volume (cm^3)

At 60 DAP, the root volume of at least three plants in three replications was measured on a per-plant basis. The 'EPSON Expression 12000XL' root scanner (Seiko Epson Corporation, Suwa-shi, Nagano-ken, Japan) was used to measure the root volume. The 'WinRHIZO Pro 2020a' program (Regent Instruments Inc., Quebec, Canada) was used to analyze the scanned pictures.

5.5.12 Root diameter (mm)

At 60 DAP, root diameter was measured per plant from a minimum of three plants in three replications. The 'EPSON Expression 12000XL' root scanner (Seiko Epson Corporation, Suwa-shi, Nagano-ken, Japan) was used to measure the root's diameter. The 'WinRHIZO Pro 2020a' program (Regent Instruments Inc., Quebec, Canada) was used to analyze the scanned pictures.

5.5.13 Tuber N content (%)

* From tuber tissues at the harvest stage (110 DAP), the total nitrogen (N) concentration in tubers (in grams per plant) was calculated on a dry weight basis. According to Singh et al. (2005), the modified Kjeldahl method was used for this. At least three plants for each trait per replication were measured.

* To put it briefly, the digestion of the plant sample in the form of a fine powder was done at 360–410 °C in sulphuric acid, in order to determine their N content. Using anhydrous sodium sulphate/potassium sulphate, the boiling temperature of H_2SO_4 was enhanced, and copper sulphate was added to be used as a catalyst to speed up the pace of digestion.

* The digestion temperature was vigilantly controlled for thorough digestion, which typically takes less than two hours. Once the samples had finished digesting, the concentrated alkali was added to the H_2SO_4 digest for distillation, and they were cooled and diluted.

* Boric acid is used to absorb the distilled ammonia quantitatively, and the results are titrated against a standard acid. Lastly, differences in potato types were examined for nitrogen usage efficiency (NUE) and its associated parameters at the harvest stage (110 DAP) using the formula of Zebarth et al., (2004, 2008).

$$\text{Nitrogen (\%)} = \frac{0.0014 \times (\text{Titre value - Blank value}) \times 100}{\text{Sample weight}}$$

Where, 0.0014 = factor (i.e. 1 ml of 0.1 N H_2SO_4 = 0.0014 g N)

5.5.14 Agronomic nitrogen use efficiency (AgNUE)

NUE physiological variables like N use efficiency (NUE), N uptake efficiency (NUpE) and N utilization efficiency (NUtE) were estimated at harvest stage (110 DAP).

$$\text{Agronomic nitrogen use efficiency (AgNUE)} = \frac{\text{Tuber yield}}{\text{Crop N supply}}$$

5.5.15 Nitrogen use efficiency (NUE)

$$\text{Nitrogen use efficiency (NUE)} = \frac{\text{Plant dry matter accumulation}}{\text{Crop N supply}}$$

5.5.16 Nitrogen uptake efficiency (NUpE)

$$\text{Nitrogen uptake efficiency (NUpE)} = \frac{\text{Plant N accumulation}}{\text{Crop N supply}}$$

5.5.17 Nitrogen utilization efficiency (NUtE)

$$\text{Nitrogen utilization efficiency (NUtE)} = \frac{\text{Plant dry matter accumulation}}{\text{Plant N accumulation}}$$

5.6 Statistical analysis

At least three plants of each of the 56 potato types were used to quantify plant biomass, yield component characteristics, root shape, and NUE parameters. All plants had their yield characteristics measured. The open source software OPSTAT was used to analyze two years' worth of data using two-way ANOVA (analysis of variance) for pooled data in a two-factor experiment of completely randomized design with Fisher's test for statistical significance ($p \leq 0.05$) (Sheoran et al., 1998). Also, the homogeneity of variance will be tested using Bartlett's Chi-square test.

5.7 Transcriptome sequencing

Selected potato varieties were used for transcriptome analysis based on the aeroponic experiments. Plant samples (leaves and tubers/stolons) were collected at the full vegetative growth stage when tuberization occurred about 60 days after planting under aeroponics growth conditions. Plant samples were kept in liquid nitrogen until they were needed for transcriptome sequencing and analysis. In order to generate transcriptome data, whole transcriptome sequencing (RNA-seq) work was outsourced to Eurofins to perform the Illumina NextSeq500 sequencing technology. A reference-based RNA-seq analysis was carried out as the potato genome is accessible.

5.7.1 Total RNA isolation and Illumina NextSeq500 paired-end (PE) library preparation

Using the Qiagen kit (QIAGEN), total RNA was extracted from the plant samples. A 1% denaturing RNA agarose gel and NanoDrop (Thermo Fisher Scientific, Wilmington, Delaware, USA) were used to evaluate the characteristics and amounts of the extracted RNA samples, respectively. Following the manufacturer's instructions, RNA-seq paired-end (PE) sequencing libraries were created from the QC-passed RNA samples using the Illumina TruSeq Stranded mRNA sample prep kit (Illumina, San Diego, CA, USA). In summary, poly-T connected magnetic beads were used to enrich mRNA from total RNA. Enzymatic fragmentation and the conversion of first-strand cDNA using SuperScript II and Act-D mix were then performed to enable RNA-dependent synthesis. The second strand mix was then used to synthesize the first strand of cDNA into the second strand. The dscDNA was then enriched by a restricted number of PCR cycles after being purified using AMPure XP beads, A-tailing, and adapter ligation. As directed by the manufacturer, the PCR-enriched libraries were examined using high-sensitivity D1000 Screen tape on the 4200 Tape Station system (Agilent Technologies, Santa Clara, CA, USA).

5.7.2 Total RNA Sequencing

The PE Illumina libraries were then loaded onto NextSeq500 for cluster creation and sequencing following the acquisition of the libraries' Qubit 3.0 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) concentrations and the mean peak sizes from Agilent Tape Station profiles. The NextSeq500 can sequence the template fragments in both forward and reverse directions thanks to paired-end sequencing. Samples were bound to complementary adapter oligos on a paired-end flow cell using the kit reagents. During sequencing, the adapters were made to enable the forward strands to be selectively cleaved, followed by the resynthesis of the reverse strand. The opposite end of the fragment was then sequenced using the reverse strands that had been copied.

5.7.3 RNA-seq data processing and high-quality read statistics

Adapter sequences, ambiguous reads (reads with unknown nucleotides "N" more than 5%), and low-quality sequences (reads with more than 10% quality threshold (QV) < 25 phred score) were eliminated from the raw data using Trimmomatic v0.38. After trimming, the nucleotide had a minimum length of 100 nt. High-quality reads were recovered from the raw data after the adaptor and low-quality sequences were removed. These high-quality (QV > 25), paired-end reads were used for reference-based read mapping. The following framework was taken into account for filtration:

i) SLIDING WINDOW

Sliding window trimming of 10 bp, cutting once the average quality within the window falls below a threshold of 25,

ii) LEADING

Cut bases off the start of a read, if below a threshold quality of 25, and

iii) TRAILING

Cut bases off the end of a read, if below a threshold quality of 25.

5.7.4 Reads mapping to the reference potato genome

The Spud DB database of the potato genome sequence provided the reference genome of Solanum tuberosum Group Phureja DM1-3, which has a genomic size of around 773 Mb and the related annotations. (http://solanaceae.plantbiology.msu.edu/index.shtml). The links for downloading the genome were

http://solanaceae.plantbiology.msu.edu/data/potato_dm_v404_all_pm_un.fasta.zip and for annotation was

http://solanaceae.plantbiology.msu.edu/data/PGSC_DM_V403_genes.gff.zip. Using TopHat v2.1.1 with default settings, the high-quality reads from the potato samples were mapped onto the previously described reference genome of *Solanum tuberosum* Group Phureja DM1-3.

5.7.5 Differentially expressed genes (DEGs) analysis

Using RNA-Seq data, the Cufflinks v2.2.1 tool compiles transcriptomes and measures their expression. Cuffdiff was utilized to do differential gene expression analysis on the individual transcriptome GTF files. The annotation file for *Solanum tuberosum* Group Phureja DM1-3 has 39,028 protein-coding genes in total. The study was done for genes that were described to be typically expressed in treated and control samples, respectively. The log fold change was computed as $\log_2$ (FPKM Experimental/FPKM Control) using FPKM values. With a P-value threshold of 0.05 for statistically significant results, Log_2 Fold Change (FC) values larger than zero were regarded as up-regulated and those less than zero as down-regulated.

5.7.6 Heatmap

Using the multiple experiments viewer, an average linkage hierarchical cluster analysis was conducted on the top 50 DEGs for each of the previously described combinations. (MeV v4.9.0). The gene abundance level is displayed in the heatmap. The $\log_2$ ratio of gene abundance between control and treatment samples is used to show expression levels. Hierarchical clustering was used to examine genes with differential expression. The log-transformed and normalized gene values based on the Pearson uncentered distance and average linkage approach were used to create heatmaps. Every horizontal line in the heatmaps represents a gene. The logarithmic intensity of the genes that are expressed is represented by the color. Red indicates expression values that are relatively high.

5.7.7 Scatter plot

Gene expression in two different regimes for every sample combination—Control and Treated—was graphically represented using the Eurofins proprietary R script. It facilitates the comparison of two gene-related values and aids in identifying genes that exhibit differential expression in one sample relative to another. A gene is represented by each dot in a scatter plot. Each gene's expression extent in the Control sample is indicated by its vertical position, but in the Treated sample, it is represented by its horizontal position. Therefore, in comparison to their median expression level in the experimental grouping of the study, genes falling above the diagonal are over-expressed and genes falling below the diagonal are under-expressed.

5.7.8 Volcano plot

The graphical representation and distribution of the differentially expressed genes present in the Control and Treated samples were shown using the Eurofins proprietary R script. The "volcano plot" organizes expressed genes according to both statistical and biological importance dimensions. Significantly down-regulated genes are represented by the green block on the left side of zero, whereas up-regulated genes are represented by the red block on the right. Data points with low p-values (very significant) appear near the top of the plot, while the Y-axis shows the negative log of the p-value (p value <0.05) of the statistical test that was run. Non-differentially expressed genes are displayed in grey blocks.

5.7.9 Gene Ontology (GO) Analysis

GO annotations were acquired from the Ensembl Plants database for Solanum tuberosum. For all 21 of the previously listed combinations, GO annotation is linked to upregulated, downregulated, expressed both, and solely expressed genes. It also provides information on the number of genes allocated to the GO Domains, which are Molecular Function (MF), Cellular Component (CC), and Biological Process (BP). The

WEGO portal was used to obtain the bar graphs that showed the GO distribution. (http://wego.genomics.org.cn/cgi-bin/wego/index.pl).

5.7.10 KEGG pathway analysis

KAAS (KEGG Automatic Annotation Server) was used to perform functional annotations of genes against the curated KEGG GENES database (http://www.genome.jp/kegg/ko.html). For route mapping, the "Nightshade" family's KEGG Orthology database served as the reference. The outcome includes automatically produced KEGG pathways utilizing the KAAS bidirectional best hit (BBH) approach against the available database, as well as KO (KEGG Orthology) designations.

5.7.11 Validation of candidate genes through quantitative real-time PCR analysis

Using an in-house machine, 'Applied Biosystems 9700HT Fast Real-Time PCR system', the selected DEGs were validated by quantitative real-time PCR (qRT-PCR) analysis. qRT-PCR was used to analyse a subset of genes from both up- and down-regulated groups in the tissues of leaves and tubers. The PrimerQuest Tool from Integrated DNA Technologies (IDT) was used to design the primers for qRT-PCR. The same RNA sample used for RNA sequencing was used to create the cDNA using the TaqMan Reverse Transcription Reagent Kit (Applied Biosystems, New Jersey, USA). The Power SYBR Green PCR Master Mix was used to create the qRT-PCR reactions using an 'ABI PRISM HT7900' (Applied Biosystems, Warrington, UK). For the reaction, the temperature and timing profiles were 50 °C for two minutes, 95 °C for ten minutes, and then 40 cycles of 95 °C for fifteen seconds, 60 °C for one minute, and 72 °C for thirty seconds. The data were analysed in triplicate using the ΔΔCt calculation method after being normalized using an internal standard, the potato ubiquitin-ribosomal protein gene (ubi3; L22576).

Chapter-6
Results and Discussion

6.1 Evaluation of potato varieties for agronomic and physio-biochemical traits under high and low N treatments in aeroponics

6.1.1 Analysis of variance (ANOVA)

Statistically significant differences (P < 0.05) were found for the variety impacts in both high N and low N in all 17 traits as a result of two-way analysis of variance (ANOVA) for two factors: variety and nitrogen (N) for pooled data of two years. (Table 6.1) *viz.*, i) plant height, ii) total leaf area, iii) total chlorophyll, iv) root dry weight, v) shoot dry weight, vi) tuber dry matter, vii) tuber no./plant, viii) tuber yield/plant, ix) root length, x) root surface area, xi) root volume, xii) root diameter, xiii) tuber N, xiv) agronomic nitrogen use efficiency (AgNUE), xv) nitrogen use efficiency (NUE), xvi) nitrogen uptake use efficiency (NUpE), and xvii) nitrogen utilization efficiency (NUtE). Similarly, the effect of nitrogen treatments (high and low N) was also statistically significant (P < 0.05) for most traits except for tuber dry matter. Moreover, varieties and nitrogen interaction were also statistically significant (P < 0.05) for all traits except agronomic nitrogen use efficiency (AgNUE) in this study. Thus, the average performance of potato types in aeroponics over a two-year period under low and high nitrogen regimes is summed up based on the above-mentioned analysis. (Tables 6.2-6.5)

6.1.2 Plant height (cm)

A significant variation (P < 0.05) was observed among the varieties, nitrogen treatments, and interaction effects for plant height. Based on the mean values of two years, Kufri Jyoti (98.77 cm) followed by Kufri Pukhraj (90.07 cm) and Kufri Frysona (86.50 cm) under high N, whereas Kufri Pukhraj (75.50 cm) followed by Kufri Jyoti (62.49 cm) and Kufri FryoM (49.75 cm) attained the maximum plant height under low N in aeroponic conditions. In contrast, varieties Kufri Safed (15.5 cm) followed by Kufri Megha (17.75 cm) under high N, and Kufri Kanchan (13.25 cm) followed by Kufri Swarna (13.50 cm) under low N achieved the lowest plant height.

6.1.3 Total leaf area (cm^2)

A significant variation (P < 0.05) was observed among the varieties, nitrogen treatments and interaction effects for total leaf area. On the basis of average values of two years, Kufri FryoM (5743.34 cm^2) followed by Kufri Badshah (4218.11 cm^2) and Kufri Chipsona-3 (2366.18 cm^2) under high N, whereas Kufri Frysona 113.17 cm^2) followed by Kufri Pukhraj (883.09 cm^2) and Kufri Khyati 37.25 cm^2) observed maximum total leaf area under low N under aeroponics. On the other hand, varieties Kufri Kumar (225.49 cm^2) followed by Kufri Surya (342.75 cm^2) under high N, while Kufri Sutlej (103.70 cm^2) and Kufri Kumar (165.03 cm^2) under low N attained the minimum total leaf area.

6.1.4 Total chlorophyll (mg/g FW)

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments and interaction effects for total chlorophyll content. Based on the average values of two years, Kufri Jyoti (2 mg/g FW) followed by Kufri Giriraj (1.98 mg/g FW) and Kufri Chipsona-3 (1.90 mg/g FW) under high N, whereas Kufri Kumar (1.49 mg/g FW) accompanied by Kufri Red (1.41 mg/g FW) and Kufri Sutlej (1.41 mg/g FW) recorded maximum total chlorophyll content under low N under aeroponics. On the contrary, varieties Kufri Kundan (0.71 mg/g FW) and Kufri Himalini (0.79 mg/g FW) under high N, while Kufri Alankar (0.47 mg/g FW) and Kufri Anand (0.58 mg/g FW) under low N attained minimum total chlorophyll content.

6.1.5 Root dry weight (g)

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments and interaction effects for root dry weight. Based on the average values for two years, Kufri Jyoti (2.01 g) followed by Kufri Pukhraj (1.43 g) and Kufri Thar-2 (1.42 g) under high N, whereas Kufri Sutlej (0.75 g) was followed by Kufri Pukhraj (0.64 g) and Kufri FryoM (0.56 g) observed maximum root dry weight under low N under aeroponics. On the other hand, varieties Kufri Sindhuri (0.01 g) followed by Kufri Megha (0.01 g) under high N, while Kufri Safed (0.04 g) and Kufri Kundan (0.06 g) under low N attained minimum root dry weight.

6.1.6 Shoot dry weight (g)

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments and interaction effects for shoot dry weight. Based on the average values of two years, Kufri FryoM (11.08 g), accompanied by Kufri Sangam (11.0 g) and Kufri Frysona (8.68 g) under high N, whereas Kufri FryoM (4.46 g), followed by Kufri Jyoti (2.28 g) and Kufri Pukhraj (1.92 g) had maximum shoot dry weight under low N under aeroponics.

On the other hand, varieties Kufri Safed (0.06 g) followed by Kufri Muthu (0.44 g) under high N, while Kufri Safed (0.06 g) and Kufri Kundan (0.14 g) under low N attained minimum shoot dry weight.

6.1.7 Tuber dry matter (%)

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments and interaction effects for tuber dry matter content. Based on the average values of two years, Kufri Thar-2 (23.31 %) followed by Kufri Dewa (21.56 %) and Kufri Red (21.36 %) under high N, whereas Kufri Swarna (30.59 %) followed by Kufri Himsona (26.08 %) and Kufri Khasigaro (25.29 %) achieved the maximum tuber dry matter content under low N under aeroponics. On the other hand, varieties Kufri Gaurav (14.39 %) followed by Kufri Sukhyati (14.46 %) under high N, while Kufri Sutlej (14.64 %) and Kufri Lalit (15.66 %) under low N attained minimum tuber dry matter content.

6.1.8 Tuber number/plant

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments and interaction effects for tuber number/plant. Based on the average values of two years, Kufri Chipsona-1 (23.55) followed by Kufri Red (20.95) and Kufri Chipsona-3 (20.89) under high N, whereas Kufri Kesar (43.52) followed by Kufri Alankar (38.32) and Kufri Ashoka (35.39) recorded maximum tuber number/plant under low N under aeroponics. On the other hand, varieties Kufri Himsona (5.34) followed by Kufri Sutlej (5.38) under high N, while Kufri Safed (2.65) and Kufri Sutlej (4.71) under low N had the minimum tuber number/plant.

6.1.9 Tuber yield/plant (g)

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments and interaction effects for tuber yield/plant. Based on the average values of two years, Kufri Frysona (96.39 g) followed by Kufri FryoM (85.39 g) and Kufri Badshah (77.87 g) under high N, whereas Kufri FryoM (60.52 g) followed by Kufri Mohan (45.10 g) and Kufri Badshah (40.36 g) observed the highest tuber yield/plant under low N under aeroponics. On the other hand, varieties Kufri Naveen (12.45 g) followed by Kufri Khasigaro (13.61 g) under high N, while Kufri Sutlej (6.59 g) and Kufri Kumar (7.60 g) under low N attained the minimum tuber yield/plant.

6.1.10 Root length (cm)

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments and interaction effects for total root length on per plant basis. Based on the average values of two years, Kufri Chipsona-3 (5969.76 cm) followed by Kufri Ashoka (5828.06 cm) and Kufri Neela (5685.04 cm) under high N, whereas Kufri Chipsona-3 (2735.24 cm) followed by Kufri Chipsona-1 (2517.50 cm) and Kufri Manik (2371.94 cm) recorded the maximum root length per plant under low N under aeroponics. On the other hand, varieties Kufri Safed (217.48 cm) followed by Kufri Kumar (457.46 cm) under high N, while Kufri Safed (275.50 cm) and Kufri Kumar (328.18 cm) under low N observed minimum root length per plant.

6.1.11 Root surface area (cm^2)

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments and interaction effects for total root surface area per plant basis. Based on the average values of two years, Kufri Chipsona-3 (699.87 cm^2) followed by Kufri Thar-2 (532.32 cm^2) and Kufri Ashoka (464.86 cm^2) under high N, whereas Kufri Chipsona-1 (191.48 cm^2) followed by Kufri Chipsona-3 (173.48 cm^2) and Kufri FryoM (162.83 cm^2) recorded maximum root surface area per plant under low N in aeroponics. On the other hand, varieties, Kufri Safed (15.75 cm^2) followed by Kufri Kumar (37.52 cm^2) under high N, while Kufri Safed (17.24 cm^2) and Kufri Kumar (30.59 cm^2) under low N recorded minimum root surface area per plant.

6.1.12 Root volume (cm^3)

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments and interaction effects for total root volume on per plant basis. Based on the average values of two years, Kufri Chipsona-3 (4.04 cm^3) followed by Kufri Thar-2 (3.62 cm^3) and Kufri Ashoka (3.48 cm^3) under high N, whereas Kufri Pukhraj (1.73 cm^3) followed by Kufri Chipsona-1 (1.18 cm^3) and Kufri Jyoti (1.11 cm^3) recorded maximum root volume per plant under low N in aeroponics. On the other hand, varieties Kufri Safed (0.04 cm^3) followed by Kufri Kumar (0.19 cm^3) under high N, while Kufri Safed (0.07 cm^3) and Kufri Kumar (0.21 cm^3) under low N recorded minimum root volume per plant.

6.1.13 Root diameter (mm)

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments and interaction effects for root diameter on a per-plant basis. Based on the average values of two years, Kufri Karan (0.44 mm) followed by Kufri Kanchan (0.29 mm) and Kufri Garima (0.29 mm) under high N, whereas Kufri Jeevan (0.31 mm) followed by Kufri Chipsona-3 (0.30 mm) and Kufri Karan (0.29 mm) recorded maximum root diameter per plant under low N in aeroponics. On the other hand, varieties Kufri Safed (0.14 mm) followed by Kufri Girdhari (0.19 mm) under high N, while Kufri Safed (0.14 mm) and Kufri Girdhari (0.19 mm) under low N recorded minimum root diameter per plant.

6.1.14 Tuber N content (%)

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments, and interaction effects for tuber N content per plant. Based on the average values of two years, Kufri Gaurav (3.07 %) followed by Kufri Red (3.03 %) and Kufri Khasigaro (2.81 %) under high N, whereas Kufri Girdhari (2.82 %) followed by Kufri Kanchan (2.81 %) and Kufri Khasigaro (2.79 %) recorded maximum tuber N content per plant under low N in aeroponics. On the other hand, varieties Kufri Pukhraj (1.14 %) followed by Kufri Karan (1.25 %) under high N, while Kufri Kundan (1.46 %) and Kufri Jeevan (1.50 %) under low N recorded minimum tuber N content per plant.

6.1.15 Agronomic nitrogen use efficiency (AgNUE)

A significant variation ($P < 0.05$) was observed among the varieties and interaction effects for agronomic nitrogen use efficiency (AgNUE). Based on the average values of two years, Kufri Frysona (0.51) followed by Kufri FryoM (0.45) and Kufri Badshah (0.41) under high N, whereas Kufri Fryom (6.05) followed by Kufri Mohan (4.38) and Kufri Badshah (4.04) recorded maximum AgNUE per plant under low N in aeroponics. On the other hand, varieties Kufri Naveen (0.07) and Kufri Khasigaro (0.08) under high N, while Kufri Kumar (0.76) and Kufri Sutlej (0.80) under low N, recorded the minimum AgNUE per plant.

6.1.16 Nitrogen use efficiency (NUE)

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments, and interaction effects for nitrogen use efficiency (NUE). Based on the average values for two years, Kufri Thar-2 (0.124) followed by Kufri Dewa (0.11) and Kufri Red (0.11) under high N, whereas Kufri Swarna (3.05) followed by Kufri Himsona (2.60) and Kufri Khasigaro (2.52) recorded maximum NUE per plant under low N in aeroponics. On the other hand, varieties Kufri Gaurav (0.07) followed by Kufri Sukhyati (0.07) under high N, while Kufri Sutlej (1.46) and Kufri Lalit (1.56) under low N recorded minimum NUE per plant.

6.1.17 Nitrogen uptake use efficiency (NUpE)

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments and interaction effects for nitrogen uptake efficiency (NUpE). Based on the average values of two years, Kufri Chamatkar (0.003) followed by Kufri Dewa (0.003) and Kufri Frysona (0.003) under high N, whereas Kufri Swarna (0.077) followed by Kufri Khasigaro (0.07) and Kufri Dewa (0.06) recorded maximum NUpE per plant under low N in aroponics. On the other hand, varieties Kufri Kumar (0.001) followed by Kufri Kuber (0.001) under high N, while Kufri Sutlej (0.023) and Kufri Ganga (0.029) under low N recorded minimum NUpE per plant.

6.1.18 Nitrogen utilization efficiency (NUtE)

A significant variation ($P < 0.05$) was observed among the varieties, nitrogen treatments and interaction effects for nitrogen utilization efficiency (NUtE). Based on the average values of two years, Kufri Pukhraj (88.10) followed by Kufri Karan (79.87) and Kufri Khyati (79.42) under high N, whereas Kufri Kundan (68.86) followed by Kufri Jeevan (66.94) and Kufri Chipsona-4 (66.43) recorded maximum NUtE per plant under low N in aroponics. On the other hand, varieties Kufri Gaurav (32.55) followed by Kufri Red (32.97) under high N, while Kufri Girdhari (35.49) and Kufri Jyoti (35.37) under low N recorded minimum NUtE per plant.

Table 6.1. Mean performance of potato varieties for two years (pooled) data on plant biomass traits under different N treatments (high N and low N) in aeroponics

Sr. No.	Variety	Plant height (cm)		Total leaf area (cm^2)		Total chlorophyll (mg/g FW)		Root dry weight (g)		Shoot dry weight (g)	
		High N	Low N	High N	Low N	High N	Low N	High N	Low N	High N	Low N
1.	Kufri Alankar	46.75	35.00	2038.70	379.18	1.21	0.47	0.40	0.20	6.10	0.88
2.	Kufri Anand	29.00	25.00	633.57	257.07	1.11	0.58	0.12	0.21	0.55	0.41
3.	Kufri Ashoka	43.00	27.75	1531.71	446.45	1.38	0.69	0.18	0.19	2.73	0.84
4.	Kufri Badshah	71.00	42.00	4218.11	624.13	1.34	0.68	0.30	0.30	5.98	0.45
5.	Kufri Bahar	35.13	24.25	799.39	299.04	1.20	0.69	0.27	0.19	2.63	0.76
6.	Kufri Chamatkar	39.25	20.25	637.81	291.69	1.45	0.89	0.17	0.12	2.34	0.29
7.	Kufri Chandramukhi	28.00	19.00	764.19	408.10	1.41	0.99	0.08	0.14	0.75	0.30
8.	Kufri Chipsona-1	52.75	29.75	1642.57	655.44	1.19	0.99	0.25	0.23	6.58	1.69
9.	Kufri Chipsona-3	58.88	35.50	2366.18	688.95	1.90	1.10	0.42	0.52	6.50	1.84
10.	Kufri Chipsona-4	47.50	40.50	1539.28	511.04	0.81	0.72	0.34	0.24	3.56	0.31
11.	Kufri Dewa	38.00	23.00	1358.79	346.21	1.13	1.22	0.18	0.14	0.58	0.34
12.	Kufri FryoM	77.00	49.75	5743.34	394.05	1.80	1.02	0.50	0.56	11.08	4.46
13.	Kufri Frysona	86.50	40.56	2126.81	1113.17	1.32	1.01	0.30	0.31	8.68	1.04

Sr. No.	Variety	Plant height (cm)		Total leaf area (cm^2)		Total chlorophyll (mg/g FW)		Root dry weight (g)		Shoot dry weight (g)	
		High N	Low N	High N	Low N	High N	Low N	High N	Low N	High N	Low N
14.	Kufri Ganga	39.50	34.25	1383.05	282.39	1.11	0.82	0.07	0.27	1.18	0.64
15.	Kufri Garima	36.50	29.00	470.40	260.24	1.26	1.17	0.23	0.27	0.94	0.44
16.	Kufri Gaurav	35.75	28.00	1334.44	456.17	0.94	0.88	0.18	0.12	1.50	0.55
17.	Kufri Girdhari	26.75	23.50	1910.69	525.39	0.93	0.76	0.21	0.14	2.37	0.44
18.	Kufri Giriraj	52.50	31.00	1575.39	428.51	1.98	0.82	0.51	0.21	3.43	0.31
19.	Kufri Himalini	37.00	21.00	1436.03	282.10	0.79	0.71	0.16	0.19	3.18	0.36
20.	Kufri Himsona	39.00	21.00	1074.49	176.58	1.72	1.41	0.36	0.06	3.23	0.25
21.	Kufri Jawahar	34.00	18.75	1978.73	706.48	1.44	0.69	0.40	0.16	4.50	0.51
22.	Kufri Jeevan	22.50	20.00	1201.77	285.36	1.10	0.79	0.38	0.20	0.90	0.44
23.	Kufri Jyoti	98.77	62.49	1212.39	727.11	2	1.33	2.01	0.53	4.99	2.28
24.	Kufri Kanchan	22.75	13.25	920.26	209.26	1.12	0.68	0.28	0.13	1.00	0.22
25.	Kufri Karan	35.50	18.50	683.47	402.37	1.20	0.69	0.29	0.20	2.25	0.15
26.	Kufri Kesar	44.25	28.75	2066.02	532.41	1.76	1.01	0.26	0.11	3.43	0.77
27.	Kufri Khyati	26.13	21.75	487.58	277.39	1.08	0.93	0.17	0.14	1.80	0.36
28.	Kufri Kuber	45.00	18.48	1377.17	737.25	1.29	0.85	0.28	0.19	3.03	1.90

Sr. No.	Variety	Plant height (cm)		Total leaf area (cm^2)		Total chlorophyll (mg/g FW)		Root dry weight (g)		Shoot dry weight (g)	
		High N	Low N	High N	Low N	High N	Low N	High N	Low N	High N	Low N
29.	Kufri Kumar	28.25	19.00	1954.09	228.71	0.81	1.02	0.28	0.19	2.20	0.29
30.	Kufri Kundan	20.50	24.75	225.49	165.04	1.13	1.49	0.19	0.10	1.73	0.29
31.	Kufri Lalima	35.50	27.00	1249.02	187.68	0.71	0.88	0.16	0.06	2.28	0.14
32.	Kufri Lalit	35.25	27.50	791.11	294.66	1.19	1.21	0.07	0.17	2.30	0.30
33.	Kufri Lauvkar	44.13	23.25	2048.32	271.34	1.12	1.00	0.16	0.11	4.19	0.89
34.	Kufri Lima	27.25	22.50	896.02	233.05	1.21	0.90	0.11	0.07	1.16	0.23
35.	Kufri Manik	29.00	25.25	1075.28	346.66	1.36	1.02	0.85	0.19	2.78	0.80
36.	Kufri Megha	41.75	24.50	1780.67	324.00	1.50	0.80	0.41	0.30	2.08	0.93
37.	Kufri Mohan	17.75	21.00	555.01	219.75	1.00	1.12	0.01	0.10	1.09	0.27
38.	Kufri Muthu	34.25	25.32	916.79	213.74	0.89	1.00	0.44	0.32	4.80	0.40
39.	Kufri Naveen	26.25	17.00	887.90	246.51	1.61	0.90	0.23	0.10	0.44	0.19
40.	Kufri Neela	27.25	19.25	600.89	166.36	1.41	1.10	0.11	0.14	1.35	0.29
41.	Kufri Neelkanth	53.75	23.50	1878.50	334.14	1.30	0.61	0.83	0.22	5.22	0.68
42.	Kufri Pukhraj	32.00	18.75	1293.00	238.51	1.11	1.09	0.07	0.19	1.31	0.31
43.	Kufri Pushkar	90.07	75.50	1120.99	883.09	1.66	1.06	1.43	0.64	2.72	1.92

Sr. No.	Variety	Plant height (cm)		Total leaf area (cm^2)		Total chlorophyll (mg/g FW)		Root dry weight (g)		Shoot dry weight (g)	
		High N	Low N	High N	Low N	High N	Low N	High N	Low N	High N	Low N
44.	Kufri Red	58.75	28.75	1908.73	515.84	1.41	1.19	0.80	0.30	5.84	1.48
45.	Kufri Sadabahar	53.50	26.25	1268.05	406.76	0.82	1.41	0.31	0.19	2.75	0.42
46.	Kufri Safed	51.50	23.50	1070.30	190.14	1.13	1.15	0.58	0.24	3.60	0.62
47.	Kufri Sahyadri	15.50	21.75	519.11	266.53	1.00	0.62	0.03	0.04	0.06	0.06
48.	Kufri Sangam	31.00	20.50	371.10	298.96	1.52	1.07	0.20	0.18	0.92	0.37
49.	Kufri Sindhuri	63.00	33.00	2307.45	338.84	1.21	1.19	1.25	0.16	11.00	0.51
50.	Kufri Sukhyati	30.80	25.00	1448.24	178.81	1.34	1.16	0.01	0.12	1.43	0.41
51.	Kufri Surya	34.38	26.50	1358.45	179.78	1.10	1.30	0.18	0.13	2.55	0.57
52.	Kufri Swarna	24.00	18.00	342.75	388.28	1.08	1.01	0.22	0.16	1.03	0.40
53.	Kufri Thar-1	39.00	14.65	1420.56	103.70	1.11	1.41	0.63	0.75	4.90	0.19
54.	Kufri Thar-2	30.50	13.50	650.39	396.39	1.05	1.29	0.36	0.13	2.36	0.29
55.	Kufri Khasigaro	50.75	22.00	1708.83	444.61	1.40	1.24	1.42	0.12	6.95	0.49
56.	Kufri Sutlej	46.00	25.00	1573.19	302.50	0.93	1.25	0.52	0.25	5.25	0.74

Mean	41.43	26.69	1388.08	376.21	1.25	0.98	0.38	0.21	3.21	0.67
CD (Var.)	6.05		153.76		0.14		0.03		1.69	
CD (N)	1.14		29.05		0.02		0.007		0.32	
CD (Var. X N)	8.56		217.45		0.19		0.05		2.39	
SE(d) (Varieties)	3.07		77.96		0.07		0.019		0.85	
SE(d) (N)	0.58		14.73		0.013		0.004		0.16	
SE(d) (Var. X N)	4.34		110.26		0.1		0.027		1.21	
C.V. (%)	7.58		8.54		6.54		10.48		11.36	

Note: CD (P < 0.05)

Table 6.2. Mean performance of potato varieties for two years (pooled) data on tuber yield traits under different N treatments (high N and low N) in aeroponics

Sr. No.	Variety	Tuber dry matter (%)		Tuber no./plant		Tuber yield/plant (g)	
		High N	Low N	High N	Low N	High N	Low N
1	Kufri Alankar	15.67	17.40	16.87	38.32	75.46	28.39
2	Kufri Anand	16.13	17.86	15.55	15.80	25.97	21.90
3	Kufri Ashoka	15.03	17.03	19.82	35.39	74.91	34.64
4	Kufri Badshah	14.63	16.67	18.45	27.80	77.87	40.36
5	Kufri Bahar	18.13	19.33	16.87	21.22	33.12	27.50
6	Kufri Chamatkar	18.72	19.50	15.91	19.70	31.05	20.81
7	Kufri Chandramukhi	19.99	21.62	16.56	11.51	21.81	18.91
8	Kufri Chipsona-1	17.63	20.30	23.55	33.48	71.98	39.06
9	Kufri Chipsona-3	18.63	21.71	20.89	28.44	60.94	37.12
10	Kufri Chipsona-4	19.28	22.90	16.53	16.54	32.02	26.55
11	Kufri Dewa	21.56	23.37	14.63	16.52	25.04	14.98
12	Kufri FryoM	18.84	18.17	16.79	29.66	85.39	60.52
13	Kufri Frysona	20.75	20.95	12.50	16.86	96.39	39.27
14	Kufri Ganga	16.84	15.90	18.60	16.69	31.79	22.95
15	Kufri Garima	16.92	20.31	10.93	12.51	23.07	19.51
16	Kufri Gaurav	14.40	17.00	18.75	18.77	40.47	30.50
17	Kufri Girdhari	19.99	21.19	13.04	18.38	48.63	22.33
18	Kufri Giriraj	16.05	20.19	12.44	23.94	61.89	22.53
19	Kufri Himalini	16.29	18.25	11.56	15.77	36.63	15.76
20	Kufri Himsona	17.87	26.08	5.35	8.18	19.42	12.12
21	Kufri Jawahar	16.08	18.66	11.45	28.82	44.98	30.38
22	Kufri Jeevan	18.90	20.12	12.07	18.04	20.34	13.97
23	Kufri Jyoti	18.45	17.53	18.64	9.97	59.26	25.15

Sr. No.	Variety	Tuber dry matter (%)		Tuber no./plant		Tuber yield/plant (g)	
		High N	Low N	High N	Low N	High N	Low N
24	Kufri Kanchan	17.04	21.84	12.86	21.70	24.00	11.55
25	Kufri Karan	17.82	20.00	18.54	23.81	21.82	19.00
26	Kufri Kesar	16.21	18.85	16.91	43.52	69.55	36.43
27	Kufri Khyati	19.69	25.29	9.43	14.11	13.61	8.05
28	Kufri Kuber	16.89	17.74	10.25	15.29	61.64	28.58
29	Kufri Kumar	16.74	24.76	12.66	15.86	17.13	17.54
30	Kufri Kundan	17.09	23.18	8.13	5.28	15.62	7.60
31	Kufri Lalima	16.80	21.89	12.50	23.56	38.48	11.45
32	Kufri Lalit	20.41	20.25	15.54	31.39	55.13	15.59
33	Kufri Lauvkar	18.97	15.66	13.50	17.82	59.42	35.59
34	Kufri Lima	16.99	19.56	10.93	11.65	20.56	13.89
35	Kufri Manik	16.96	18.52	11.64	19.89	46.93	18.13
36	Kufri Megha	16.16	17.97	7.84	12.89	37.47	18.85
37	Kufri Mohan	18.84	21.77	13.71	8.50	14.11	11.59
38	Kufri Muthu	15.07	16.42	20.57	22.71	58.87	45.10
39	Kufri Naveen	17.98	19.84	8.08	13.08	20.24	9.59
40	Kufri Neela	20.89	22.26	11.77	13.64	12.46	10.70
41	Kufri Neelkanth	14.52	21.00	11.03	14.50	32.23	16.28
42	Kufri Pukhraj	19.08	19.39	11.93	16.39	44.81	15.87
43	Kufri Pushkar	15.65	16.66	16.06	13.20	59.18	30.60
44	Kufri Red	16.14	17.46	17.03	28.29	62.51	32.49
45	Kufri Sadabahar	21.36	24.49	20.95	27.39	26.00	17.04
46	Kufri Safed	18.13	17.29	9.31	18.04	36.97	16.53
47	Kufri Sahyadri	16.26	17.30	5.75	2.65	29.58	15.50
48	Kufri Sangam	16.94	21.82	13.04	13.86	19.34	17.01
49	Kufri Sindhuri	14.93	18.20	9.94	21.96	58.71	30.38

Sr. No.	Variety	Tuber dry matter (%)		Tuber no./plant		Tuber yield/plant (g)	
		High N	Low N	High N	Low N	High N	Low N
50	Kufri Sukhyati	19.17	23.38	12.23	22.07	23.74	8.38
51	Kufri Surya	14.47	16.55	13.57	22.34	36.00	18.40
52	Kufri Swarna	18.09	22.04	15.32	15.38	20.45	17.72
53	Kufri Thar-1	17.09	14.64	5.38	4.71	36.51	6.59
54	Kufri Thar-2	16.13	30.59	8.57	10.66	34.12	18.67
55	Kufri Khasigaro	23.31	21.92	9.44	13.43	37.33	14.43
56	Kufri Sutlej	20.95	20.14	13.84	22.98	33.90	18.35
	Mean	17.67	20.01	13.68	19.01	40.66	22.12
	CD (Var.)	2.30		5.43		5.01	
	CD (N)	0.43		1.02		0.94	
	CD (Var. X N)	3.26		7.68		7.08	
	SE(d) (Varieties)	1.16		2.75		2.54	
	SE(d) (N)	0.22		0.52		0.48	
	SE(d) (Var. X N)	1.65		3.89		3.59	
	C.V. (%)	11.2		8.83		13.25	

Note: CD ($P < 0.05$)

Table 6.3. Mean performance of potato varieties for two years (pooled) data on root morphology traits under different N treatments (high N and low N) in aeroponics

Sr. No.	Variety	Root length (cm)		Root surface area (cm^2)		Root volume (cm^3)		Root diameter (mm)	
		High N	Low N	High N	Low N	High N	Low N	High N	Low N
1	Kufri Alankar	4,905.08	1,442.62	368.56	93.75	2.27	0.50	0.25	0.20
2	Kufri Anand	1,300.21	878.68	97.18	60.73	0.53	0.30	0.20	0.21
3	Kufri Ashoka	5,828.06	1,499.53	464.87	89.48	3.48	0.49	0.28	0.20
4	Kufri Badshah	5,234.03	2,103.83	390.88	118.54	2.40	0.59	0.24	0.18
5	Kufri Bahar	2,203.05	2,296.26	187.55	98.96	1.23	0.64	0.27	0.20
6	Kufri Chamatkar	1,144.99	1,068.04	80.23	80.09	0.38	0.40	0.21	0.20
7	Kufri Chandramukhi	665.13	475.74	59.10	38.47	0.30	0.21	0.23	0.21
8	Kufri Chipsona-1	3,354.23	2,517.50	255.16	191.49	1.43	1.18	0.24	0.23
9	Kufri Chipsona-3	5,969.76	2,735.24	699.87	173.48	4.04	1.03	0.22	0.30
10	Kufri Chipsona-4	1,786.88	1,438.35	134.36	91.49	0.65	0.43	0.23	0.20
11	Kufri Dewa	1,198.40	1,741.12	100.78	125.89	0.65	0.71	0.27	0.23
12	Kufri FryoM	3,410.76	2,003.88	257.78	162.83	1.54	0.74	0.26	0.22
13	Kufri Frysona	4,106.09	1,889.46	181.47	136.73	2.09	0.83	0.23	0.22

Sr. No.	Variety	Root length (cm)		Root surface area (cm^2)		Root volume (cm^3)		Root diameter (mm)	
		High N	Low N	High N	Low N	High N	Low N	High N	Low N
14	Kufri Ganga	1,986.66	1,122.56	111.48	94.50	0.81	0.62	0.25	0.27
15	Kufri Garima	2,062.93	1,788.38	199.97	140.60	1.43	0.97	0.29	0.23
16	Kufri Gaurav	3,454.42	1,346.08	253.95	99.78	1.64	0.56	0.28	0.24
17	Kufri Girdhari	1,888.98	1,691.74	119.55	126.04	0.61	0.74	0.19	0.25
18	Kufri Giriraj	2,372.60	939.29	186.66	69.78	1.16	0.39	0.26	0.21
19	Kufri Himalini	1,779.77	1,288.92	119.78	82.62	0.75	0.46	0.26	0.22
20	Kufri Himsona	960.88	931.96	78.62	62.41	0.49	0.33	0.26	0.21
21	Kufri Jawahar	4,777.49	1,948.50	377.94	143.94	2.29	0.66	0.26	0.23
22	Kufri Jeevan	1,415.90	497.99	99.85	44.01	0.53	0.31	0.23	0.32
23	Kufri Jyoti	2,082.62	946.43	213.60	105.34	2.31	1.11	0.21	0.27
24	Kufri Kanchan	878.19	950.91	92.55	67.82	0.58	0.35	0.30	0.23
25	Kufri Karan	945.79	1,184.59	121.03	106.04	1.33	0.74	0.44	0.29
26	Kufri Kesar	3,033.73	998.84	294.06	70.29	2.82	0.39	0.26	0.23
27	Kufri Khyati	997.60	1,104.88	81.00	70.63	0.45	0.38	0.25	0.25
28	Kufri Kuber	1,787.41	1,423.39	230.15	97.40	3.23	0.53	0.26	0.21

Sr. No.	Variety	Root length (cm)		Root surface area (cm^2)		Root volume (cm^3)		Root diameter (mm)	
		High N	Low N	High N	Low N	High N	Low N	High N	Low N
29	Kufri Kumar	2,856.73	589.80	175.38	48.51	0.85	0.29	0.22	0.23
30	Kufri Kundan	457.46	328.18	37.52	30.59	0.19	0.21	0.22	0.27
31	Kufri Lalima	2,896.67	1,286.92	227.25	89.39	1.39	0.43	0.23	0.22
32	Kufri Lalit	2,509.63	831.44	178.96	63.30	1.05	0.38	0.23	0.24
33	Kufri Lauvkar	4,499.12	1,200.16	348.29	79.76	1.85	0.47	0.27	0.21
34	Kufri Lima	2,498.11	1,251.96	153.72	85.39	0.82	0.45	0.20	0.24
35	Kufri Manik	3,048.34	1,588.26	257.59	117.96	1.74	0.78	0.25	0.26
36	Kufri Megha	2,063.30	2,371.94	149.40	152.39	0.72	0.76	0.19	0.22
37	Kufri Mohan	1,294.43	999.05	96.98	64.22	0.54	0.31	0.24	0.21
38	Kufri Muthu	3,779.05	2,092.57	310.99	107.17	1.81	0.44	0.25	0.16
39	Kufri Naveen	1,648.87	844.49	132.76	61.67	0.75	0.34	0.24	0.21
40	Kufri Neela	1,479.79	703.84	133.47	63.14	0.80	0.41	0.25	0.26
41	Kufri Neelkanth	5,685.04	906.06	396.62	62.92	2.20	0.29	0.20	0.23
42	Kufri Pukhraj	2,009.63	1,847.15	151.90	107.34	0.84	0.51	0.21	0.21
43	Kufri Pushkar	1,642.74	1,278.11	195.99	148.48	2.32	1.73	0.23	0.21

Sr. No.	Variety	Root length (cm)		Root surface area (cm^2)		Root volume (cm^3)		Root diameter (mm)	
		High N	Low N	High N	Low N	High N	Low N	High N	Low N
44	Kufri Red	2,383.01	1,703.39	195.25	131.15	1.29	0.84	0.22	0.25
45	Kufri Sadabahar	1,144.17	1,454.70	81.67	89.68	0.41	0.44	0.21	0.19
46	Kufri Safed	3,275.53	720.79	278.98	54.75	1.84	0.31	0.24	0.23
47	Kufri Sahyadri	217.49	275.50	15.75	17.24	0.04	0.07	0.14	0.14
48	Kufri Sangam	1,439.12	842.41	92.79	57.73	0.49	0.31	0.20	0.22
49	Kufri Sindhuri	3,256.86	1,204.93	325.34	77.81	2.75	0.37	0.24	0.19
50	Kufri Sukhyati	1,112.32	714.76	85.69	50.97	0.43	0.31	0.21	0.23
51	Kufri Surya	1,931.96	1,212.63	119.19	77.40	0.68	0.40	0.20	0.22
52	Kufri Swarna	3,517.88	1,063.40	234.75	89.26	1.24	0.54	0.21	0.23
53	Kufri Thar-1	1,249.90	868.39	107.44	72.47	0.62	0.36	0.23	0.20
54	Kufri Thar-2	2,250.01	764.51	154.17	54.38	0.87	0.31	0.24	0.20
55	Kufri Khasigaro	1,168.47	876.29	532.33	66.27	3.62	0.39	0.23	0.23
56	Kufri Sutlej	1,201.76	1,236.74	93.63	70.04	0.59	0.34	0.25	0.19

Mean	2,393.73	1,273.45	198.60	89.90	1.32	0.52	0.24	0.22
CD (Var.)	1,038.57		106.893		0.746		0.031	
CD (N)	196.271		20.201		0.141		0.006	
CD (Var. X N)	1,468.76		151.169		1.056		0.044	
SE(d) (Varieties)	526.632		54.203		0.379		0.016	
SE(d) (N)	99.524		10.243		0.072		0.003	
SE(d) (Var. X N)	744.77		76.654		0.535		0.022	
C.V. (%)	18.24		25.85		17.24		16.58	

Note: CD (P < 0.05)

Table 6.4. Mean performance of potato varieties for two years (pooled) data on Nitrogen Use Efficiency (NUE) parameters under different N treatments (high N and low N) in aeroponics

Sr. No.	Variety	Tuber N content (%)		AgNUE		NUE		NUpE		NUtE	
		High N	Low N	High N	Low N	High N	Low N	High N	Low N	High N	Low N
1	Kufri Alankar	2.43	1.82	0.40	2.84	0.083	1.740	0.002	0.032	41.70	55.11
2	Kufri Anand	2.23	1.68	0.14	2.19	0.086	1.786	0.002	0.030	45.14	59.53
3	Kufri Ashoka	2.09	1.98	0.40	3.47	0.079	1.702	0.002	0.034	47.86	50.58
4	Kufri Badshah	2.06	2.04	0.41	4.04	0.078	1.667	0.002	0.034	48.65	49.24
5	Kufri Bahar	2.11	1.94	0.18	2.75	0.096	1.933	0.002	0.038	47.52	51.62
6	Kufri Chamatkar	2.54	2.25	0.17	2.08	0.099	1.950	0.003	0.044	39.42	44.79
7	Kufri Chandramukhi	1.67	2.40	0.12	1.89	0.106	2.162	0.002	0.052	59.93	41.96
8	Kufri Chipsona-1	1.87	2.36	0.38	3.91	0.093	2.030	0.002	0.048	53.50	42.44
9	Kufri Chipsona-3	2.36	2.68	0.32	3.71	0.098	2.171	0.002	0.058	42.35	37.39
10	Kufri Chipsona-4	2.02	1.51	0.17	2.66	0.102	2.290	0.002	0.034	49.60	66.43
11	Kufri Dewa	2.27	2.68	0.13	1.50	0.114	2.337	0.003	0.063	44.06	37.40
12	Kufri FryoM	2.40	2.01	0.45	6.05	0.100	1.817	0.002	0.037	41.63	49.87
13	Kufri Frysona	2.47	2.66	0.51	3.64	0.110	2.095	0.003	0.056	40.49	37.71

Sr. No.	Variety	Tuber N content (%)		AgNUE		NUE		NUpE		NUtE	
		High N	Low N	High N	Low N	High N	Low N	High N	Low N	High N	Low N
14	Kufri Ganga	2.39	1.80	0.17	2.30	0.089	1.590	0.002	0.029	41.89	55.58
15	Kufri Garima	2.07	2.57	0.12	1.95	0.090	2.031	0.002	0.052	48.41	38.99
16	Kufri Gaurav	3.07	2.02	0.22	3.05	0.076	1.700	0.002	0.034	32.55	49.54
17	Kufri Girdhari	2.18	2.82	0.26	2.23	0.106	2.119	0.002	0.060	45.85	35.49
18	Kufri Giriraj	2.18	1.66	0.33	2.25	0.085	2.019	0.002	0.034	45.91	60.48
19	Kufri Himalini	1.81	1.76	0.20	1.58	0.086	1.825	0.002	0.032	55.18	56.92
20	Kufri Himsona	1.51	2.14	0.11	1.21	0.095	2.608	0.001	0.056	66.14	46.83
21	Kufri Jawahar	1.81	1.85	0.24	3.04	0.085	1.866	0.002	0.035	55.41	54.13
22	Kufri Jeevan	2.23	1.50	0.11	1.40	0.100	2.012	0.002	0.030	44.95	66.94
23	Kufri Jyoti	2.38	2.77	0.32	2.58	0.010	1.750	0.002	0.048	41.49	35.57
24	Kufri Kanchan	2.09	2.81	0.13	1.16	0.090	2.184	0.002	0.061	48.02	35.60
25	Kufri Karan	1.25	2.60	0.12	1.90	0.094	2.000	0.001	0.052	79.88	38.51
26	Kufri Kesar	1.68	2.37	0.37	3.64	0.086	1.885	0.001	0.045	59.53	42.20
27	Kufri Khyati	2.81	2.79	0.08	0.81	0.104	2.529	0.003	0.070	35.59	36.36
28	Kufri Kuber	1.26	2.71	0.33	2.95	0.089	1.774	0.001	0.049	79.42	37.03

Sr. No.	Variety	Tuber N content (%)		AgNUE		NUE		NUpE		NUtE	
		High N	Low N	High N	Low N	High N	Low N	High N	Low N	High N	Low N
29	Kufri Kumar	1.62	2.41	0.09	1.75	0.089	2.476	0.001	0.059	63.55	41.57
30	Kufri Kundan	1.63	2.66	0.09	0.76	0.090	2.318	0.001	0.061	62.01	37.81
31	Kufri Lalima	2.23	1.46	0.20	1.15	0.089	2.188	0.002	0.032	45.08	68.86
32	Kufri Lalit	1.54	2.05	0.30	1.56	0.108	2.025	0.002	0.041	65.01	48.97
33	Kufri Lauvkar	1.51	2.68	0.32	3.56	0.100	1.566	0.002	0.042	66.45	37.49
34	Kufri Lima	1.86	2.64	0.11	1.39	0.090	1.956	0.002	0.052	53.86	38.10
35	Kufri Manik	1.57	2.61	0.25	1.81	0.090	1.852	0.001	0.048	63.85	38.53
36	Kufri Megha	1.68	2.66	0.20	1.89	0.086	1.797	0.001	0.048	59.54	38.00
37	Kufri Mohan	1.41	1.85	0.08	1.16	0.100	2.177	0.001	0.040	70.82	54.95
38	Kufri Muthu	1.98	2.36	0.31	4.39	0.080	1.642	0.002	0.039	50.46	43.05
39	Kufri Naveen	1.82	2.64	0.11	0.96	0.095	1.984	0.002	0.052	55.11	38.25
40	Kufri Neela	1.83	2.72	0.07	1.07	0.111	2.226	0.002	0.060	54.81	37.15
41	Kufri Neelkanth	2.20	2.16	0.17	1.63	0.077	2.100	0.002	0.045	45.46	46.55
42	Kufri Pukhraj	1.66	2.28	0.24	1.59	0.101	1.939	0.002	0.045	60.28	45.12
43	Kufri Pushkar	1.14	2.61	0.30	3.23	0.200	2.170	0.001	0.058	88.10	38.60

Sr. No.	Variety	Tuber N content (%)		AgNUE		NUE		NUpE		NUtE	
		High N	Low N	High N	Low N	High N	Low N	High N	Low N	High N	Low N
44	Kufri Red	1.42	2.31	0.33	3.25	0.085	1.746	0.001	0.040	70.70	43.73
45	Kufri Sadabahar	3.04	2.54	0.14	1.70	0.113	2.449	0.003	0.062	32.97	39.81
46	Kufri Safed	2.00	2.22	0.20	1.65	0.096	1.729	0.002	0.038	50.14	45.42
47	Kufri Sahyadri	2.06	2.65	0.16	1.55	0.086	1.730	0.002	0.046	48.54	37.90
48	Kufri Sangam	1.37	2.48	0.10	1.70	0.090	2.182	0.001	0.054	73.12	40.43
49	Kufri Sindhuri	1.69	2.32	0.31	3.04	0.079	1.819	0.001	0.042	59.19	43.32
50	Kufri Sukhyati	2.13	2.71	0.13	0.84	0.101	2.338	0.002	0.063	47.08	37.11
51	Kufri Surya	1.99	2.42	0.19	1.84	0.076	1.655	0.002	0.041	50.26	43.14
52	Kufri Swarna	1.86	2.36	0.11	1.77	0.096	2.204	0.002	0.052	53.86	42.54
53	Kufri Thar-1	2.51	1.55	0.19	0.80	0.090	1.464	0.002	0.023	39.84	64.79
54	Kufri Thar-2	2.22	2.65	0.18	1.87	0.085	3.059	0.002	0.077	45.00	38.94
55	Kufri Khasigaro	2.00	2.30	0.20	1.44	0.124	2.191	0.003	0.051	49.95	43.72
56	Kufri Sutlej	2.05	2.38	0.18	1.84	0.111	2.014	0.003	0.048	50.38	43.18

	Mean	1.99	2.30	0.22	2.21	0.094	2.010	0.002	0.047	52.81	45.20
	CD (Var.)	0.129		0.303		0.229		0.005		2.49	
	CD (N)	0.024		0.057		0.043		0.001		0.47	
	CD (Var. X N)	0.183		0.428		0.325		0.008		3.53	
	SE(d) (Varieties)	0.066		0.153		0.116		0.003		1.26	
	SE(d) (N)	0.012		0.029		0.022		0.001		0.24	
	SE(d) (Var. X N)	0.093		0.217		0.165		0.004		1.79	
	C.V. (%)	7.45		9.26		3.89		8.93		6.45	

Note: CD ($P < 0.05$)

Table 6.5. Two-way ANOVA analysis for varieties and N treatments for pooled data of two years

		Plant height (cm)		Total leaf area (cm^2)		Total chlorophyll (mg/g FW)		Root dry weight (g)	
Source of Variation	**DF**	**Sum of Squares**	**Mean Squares**	**Sum of Squares**	**Mean Squares**	**Sum of Squares**	**Mean Squares**	**Sum of Squares**	**Mean Squares**
Replication	2	392.568		1,38,835.56		0.306		0.021	
Varieties	55	64,672.62	1175.866**	8,01,93,709.97	1458067.454**	9.485	0.172**	18.162	0.33**
Nitrogen	1	17,246.80	17246.796**	8,57,68,825.10	85768825.103**	7.973	7.973**	2.295	2.295**
Intraction Var X N	55	8,076.58	146.847**	5,98,74,223.25	1088622.241**	8.291	0.151**	9.211	0.167**
Error	222	6,281.46	28.295	40,48,593.14	18,236.91	3.345	0.015	0.242	0.001
Total	335	96,670.03		23,00,24,187.01		29.401		29.93	

Table 6.5 Continue…

		Shoot dry weight (g)		Tuber dry matter (%)		Tuber no./plant		Tuber yield/plant (g)	
Source of Variation	**DF**	**Sum of Squares**	**Mean Squares**	**Sum of Squares**	**Mean Squares**	**Sum of Squares**	**Mean Squares**	**Sum of Squares**	**Mean Squares**
Replication	2	21.377		5.246		386.876		558.589	
Varieties	55	759.238	13.804**	1,438.60	26.156**	10,597.63	192.684**	74,119.86	1347.634**
Nitrogen	1	559.863	559.863**	495.603	495.603	2,863.80	2863.804**	28,679.49	28679.486**
Intraction Var X N	55	396.307	7.206**	681.897	12.398**	3,153.83	57.342**	14,790.00	268.909**
Error	222	489.883	2.207	910.494	4.101	5,055.70	22.773	4,298.16	19.361
Total	335	2,226.67		3,531.84		22,057.83		1,22,446.10	

Table 6.5 Continue…

		Root length (cm)		Root surface area (cm^2)		Root volume (cm^3)		Root diameter (mm)	
Source of Variation	**DF**	**Sum of Squares**	**Mean Squares**	**Sum of Squares**	**Mean Squares**	**Sum of Squares**	**Mean Squares**	**Sum of Squares**	**Mean Squares**
Replication	2	18,55,497.86		80,083.26		5.993		0.002	
Varieties	55	25,89,11,972.51	4707490.409**	18,89,437.60	34353.411**	100.717	1.831**	0.254	0.005**
Nitrogen	1	10,43,40,538.52	104340538.521**	10,06,599.72	1006599.724**	52.518	52.518**	0.016	0.016**
Intraction Var X N	55	12,51,91,066.23	2276201.204**	12,13,509.62	22063.811**	62.42	1.135**	0.155	0.003**
Error	222	18,47,09,246.75	8,32,023.63	19,56,666.29	8,813.81	95.417	0.43	0.165	0.001
Total	335	67,50,08,321.87		61,46,296.48		317.066		0.593	

Table 6.5 Continue…

		Tuber N (%)		Agronomic Nitrogen Use Efficiency (AgNUE)		Nitrogen Use Efficiency (NUE)		Nitrogen Uptake Efficiency (NUpE)	
Source of Variation	**DF**	**Sum of Squares**	**Mean Squares**	**Sum of Squares**	**Mean Squares**	**Sum of Squares**	**Mean Squares**	**Sum of Squares**	**Mean Squares**
Replication	2	0.649		0.325		0.077		0	
Varieties	55	22.361	0.407**	111.109	2.02**	7.424	0.135**	0.011	0**
Nitrogen	1	8.24	8.24**	335.404	335.404**	308.597	308.597**	0.167	0.167**
Intraction Var X N	55	31.868	0.579**	79.886	1.452	7.025	0.128**	0.011	0**
Error	222	2.872	0.013	15.674	0.071	9.018	0.041	0.005	0
Total	335	65.99		542.399		332.141		0.195	

Table 6.5 Continue…

		Nitrogen Utilization Efficiency (NUtE)	
Source of Variation	**DF**	**Sum of Squares**	**Mean Squares**
Replication	2	195.893	
Varieties	55	14,085.46	256.099**
Nitrogen	1	4,856.20	4856.201**
Intraction Var X N	55	22,045.46	400.827**
Error	222	1,064.18	4.794
Total	335	42,247.19	

Fig. 6.1. Evaluation of potato varieties under high N and low N treatments under aeroponics (early stage)

Fig. 6.2. Evaluation of potato varieties under high N and low N treatments under aeroponics (full crop stage)

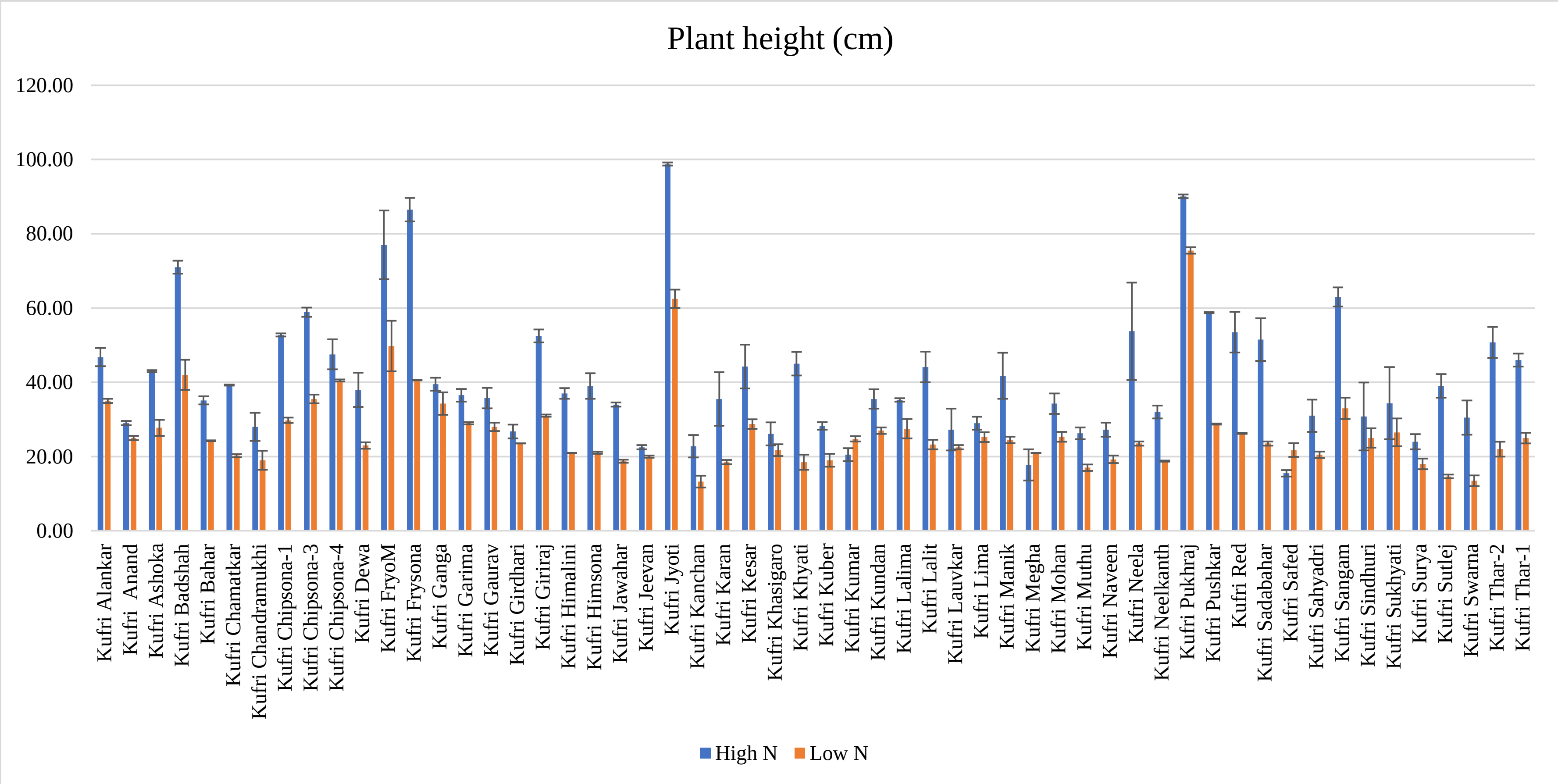

Fig. 6.3.1. Graph depicting plant height of 56 potato varieties under high & low N regimes.

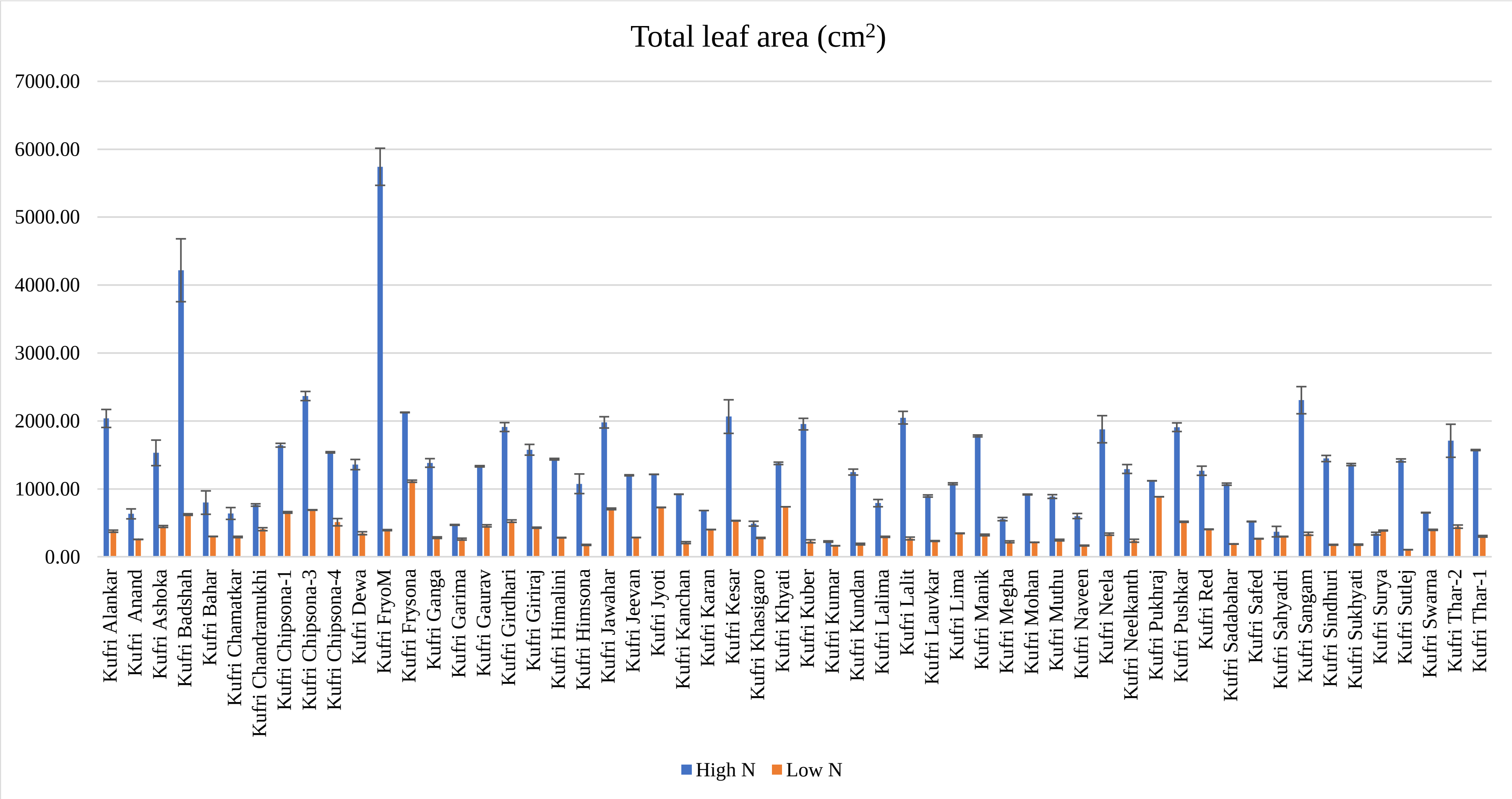

Fig 6.3.2. Graph depicting the total leaf area of 56 potato varieties under high & low N regimes.

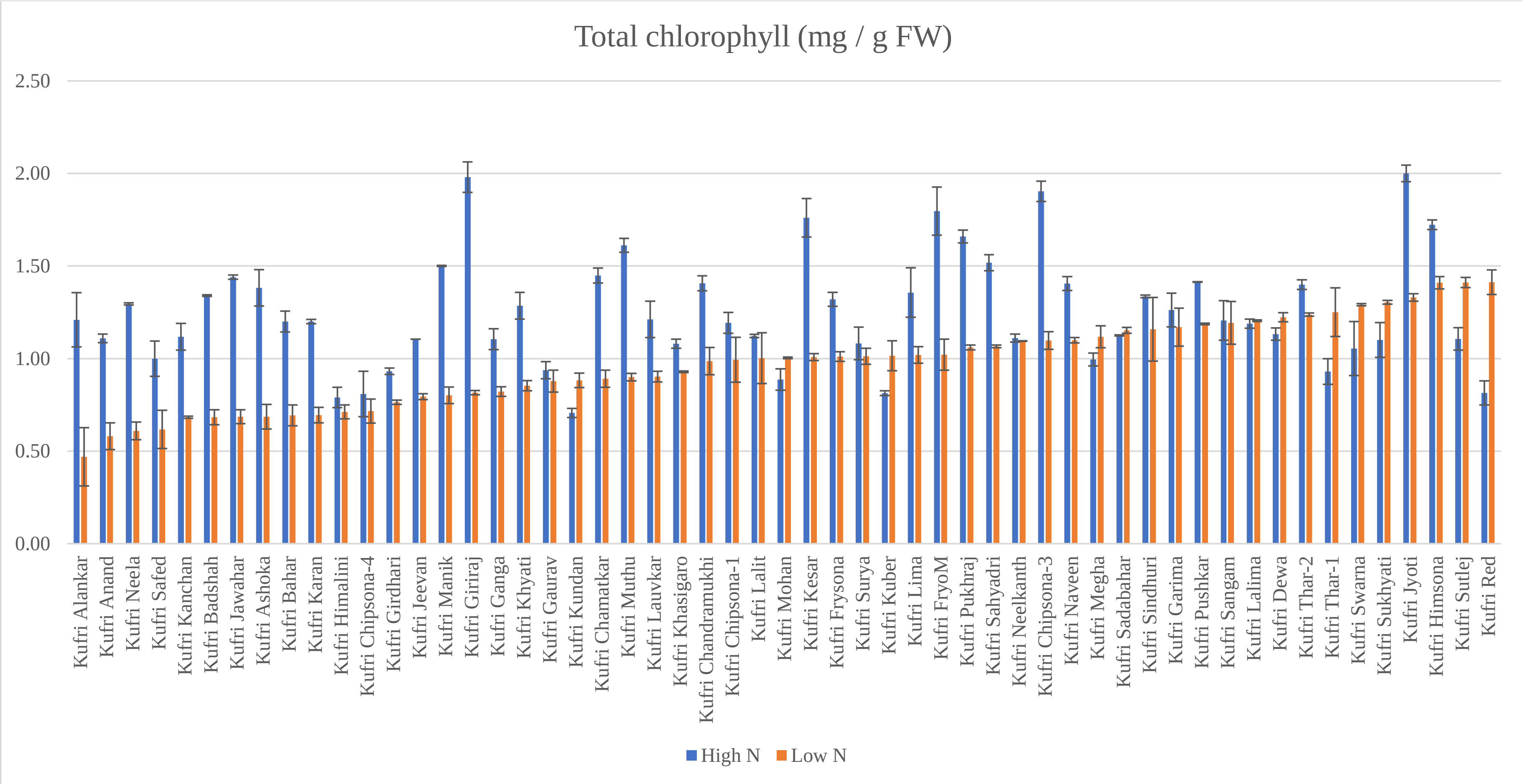

Fig 6.3.3. Graph depicting total chlorophyll of 56 potato varieties under high & low N regimes.

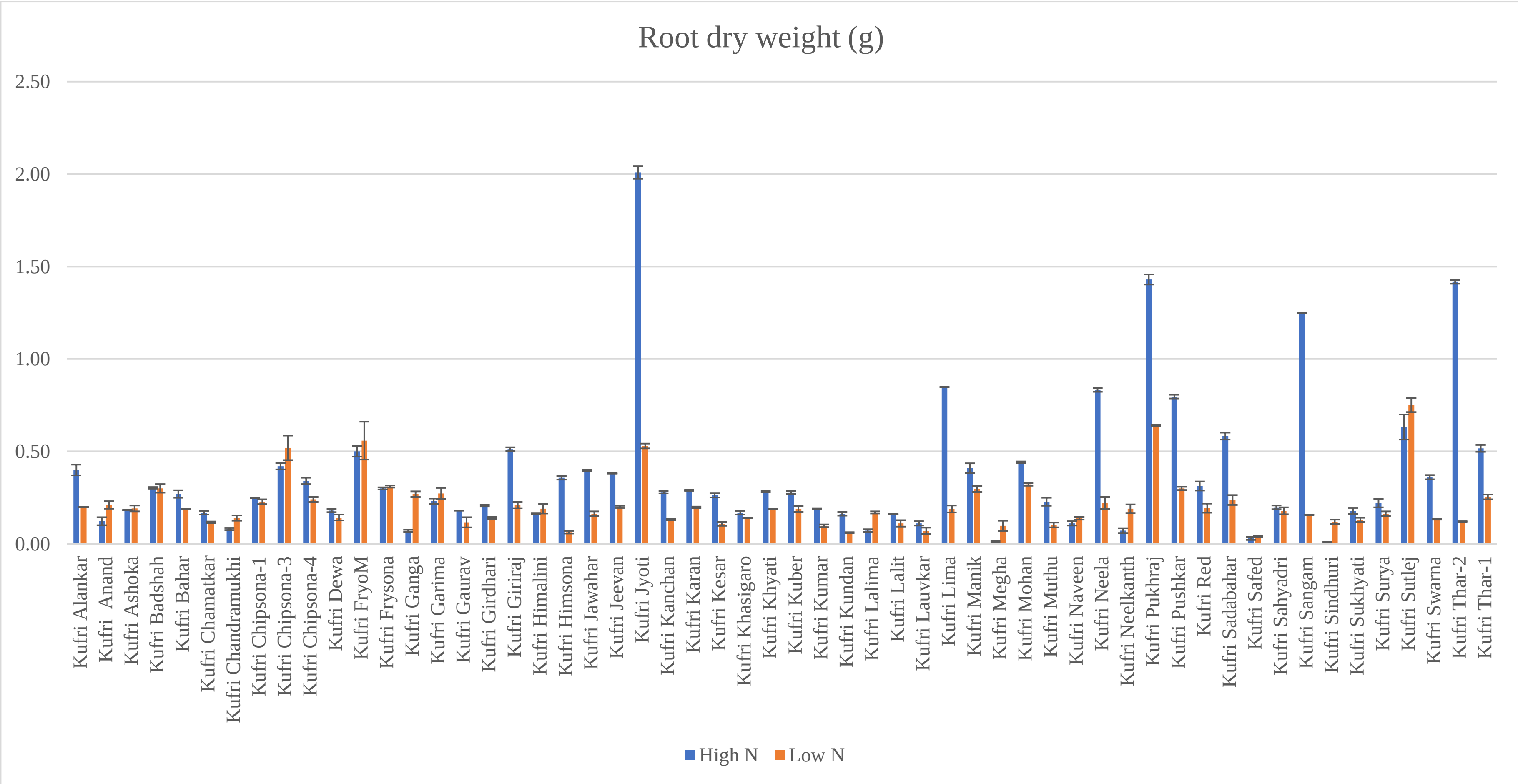

Fig 6.3.4. Graph depicting the root dry weight of 56 potato varieties under high & low N regimes.

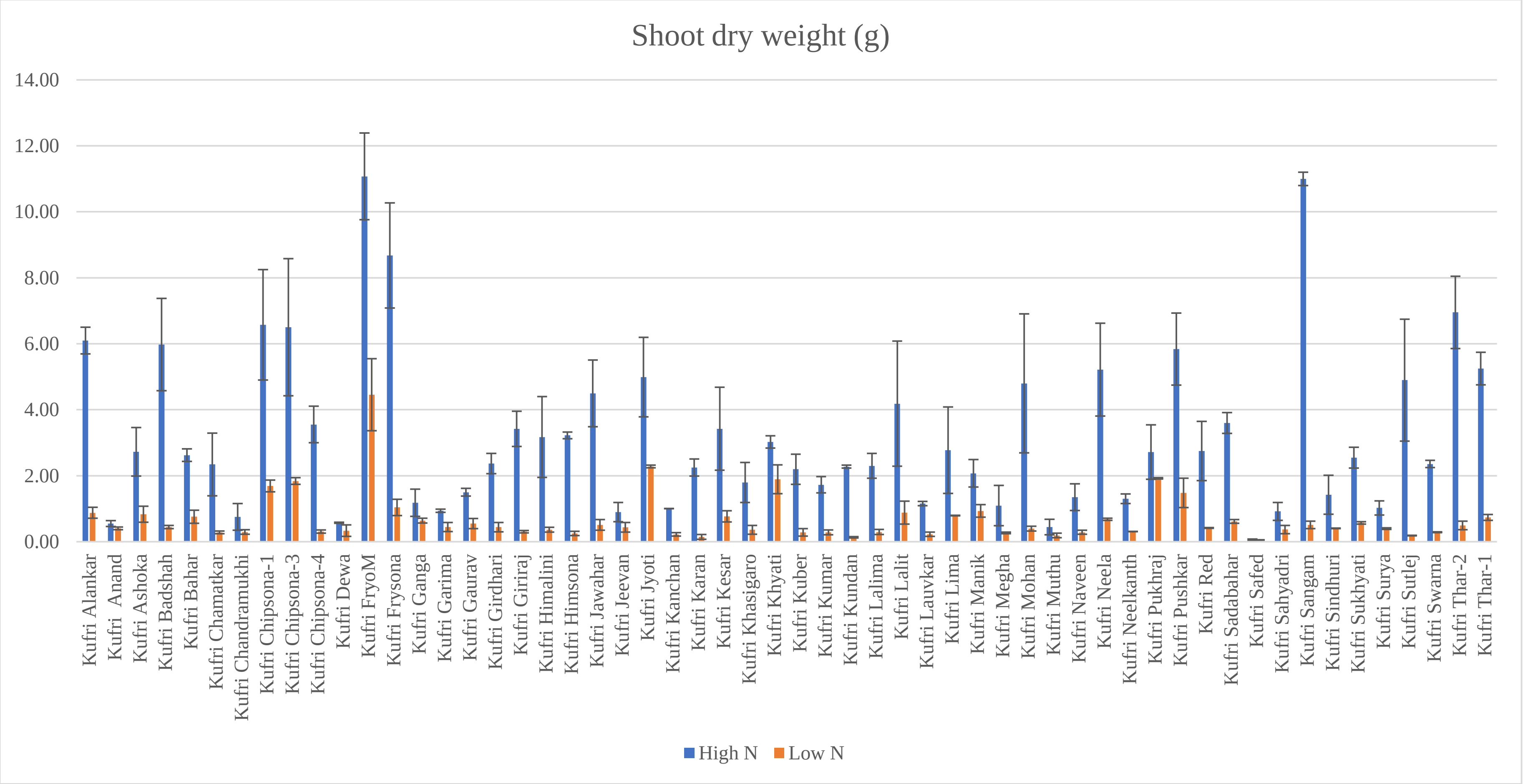

Fig 6.3.5. Graph depicting the shoot dry weight of 56 potato varieties under high & low N regimes.

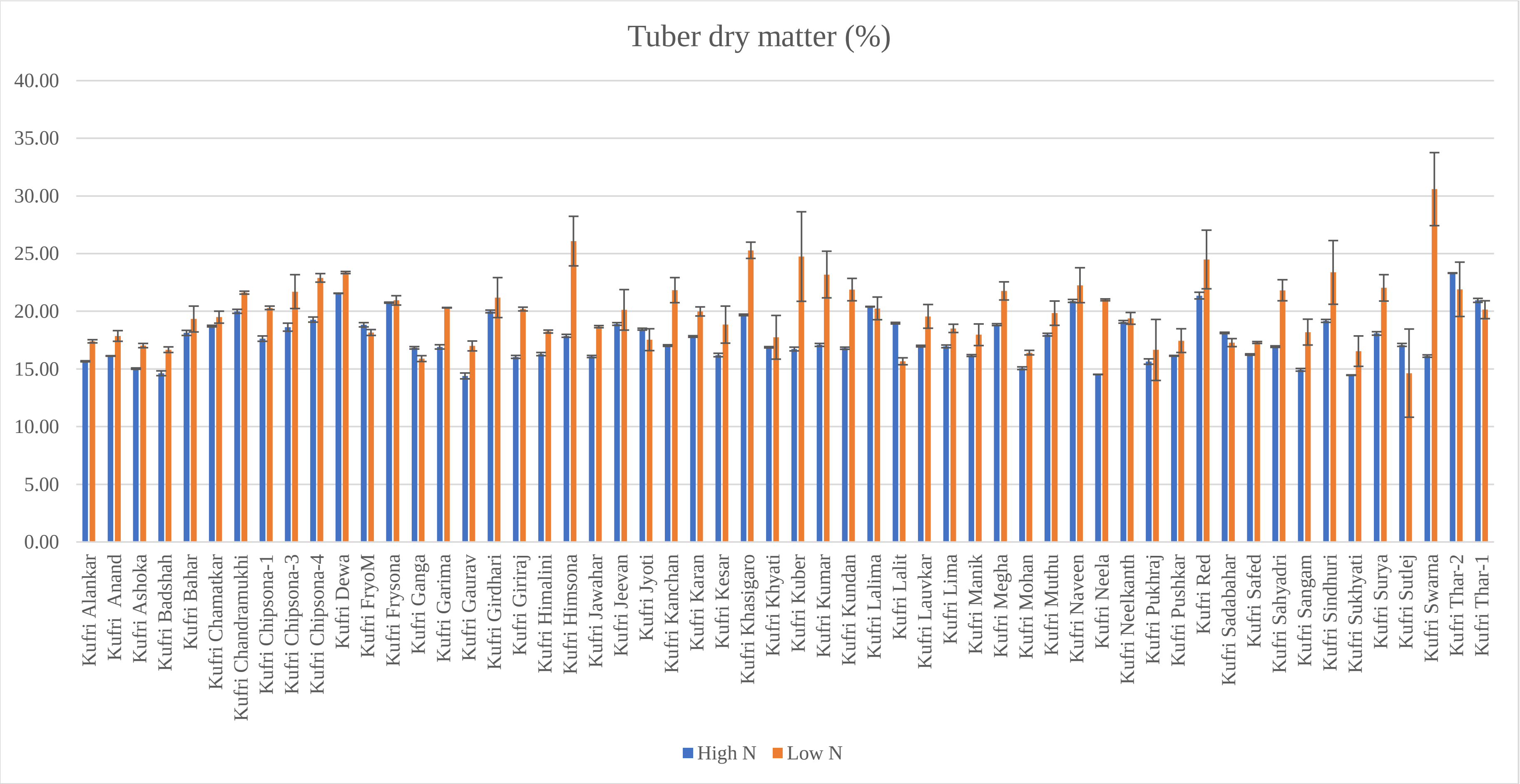

Fig 6.3.6. Graph depicting tuber dry matter of 56 potato varieties under high & low N regimes.

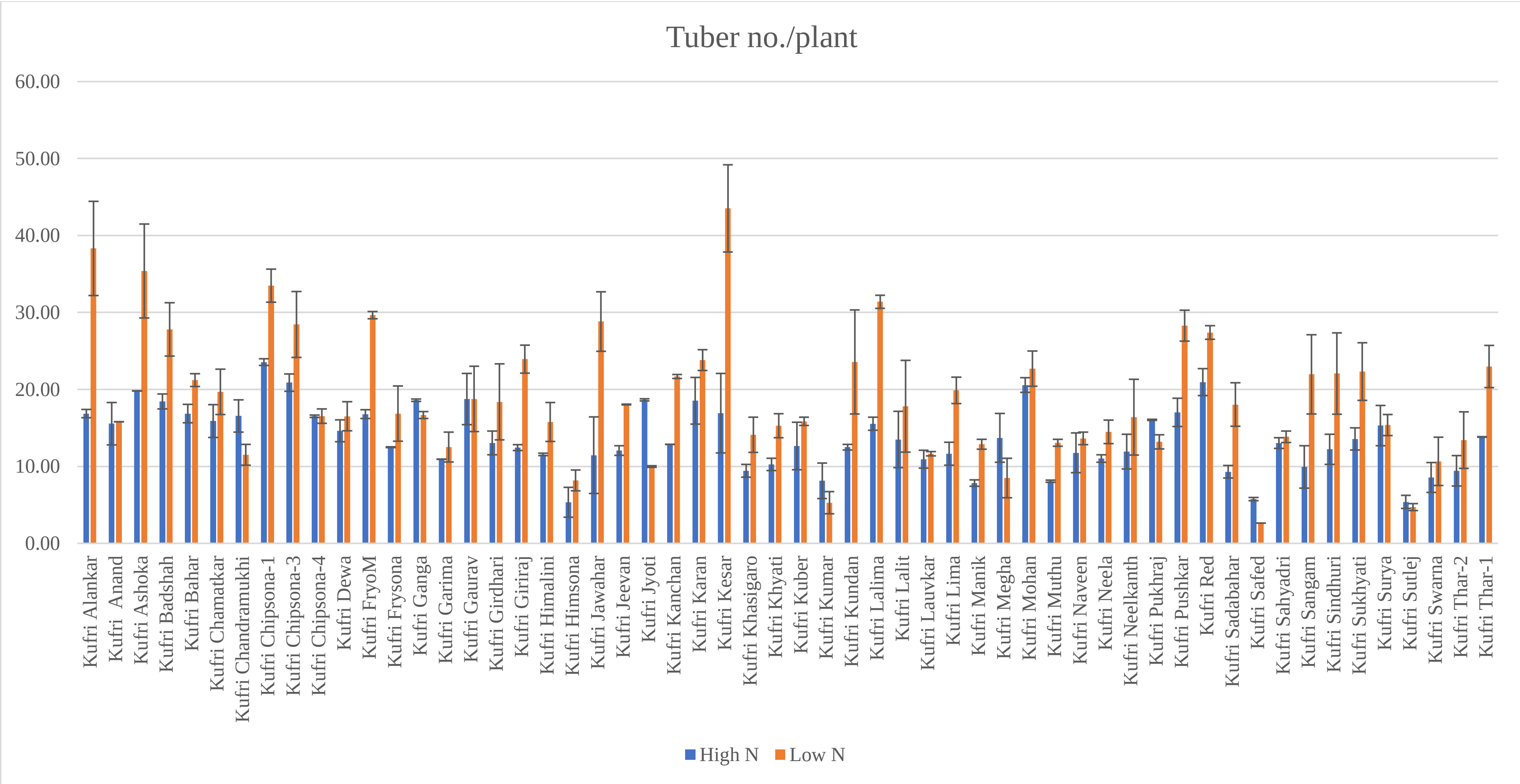

Fig 6.3.7. Graph depicting tuber number per plant of 56 potato varieties under high & low N regimes.

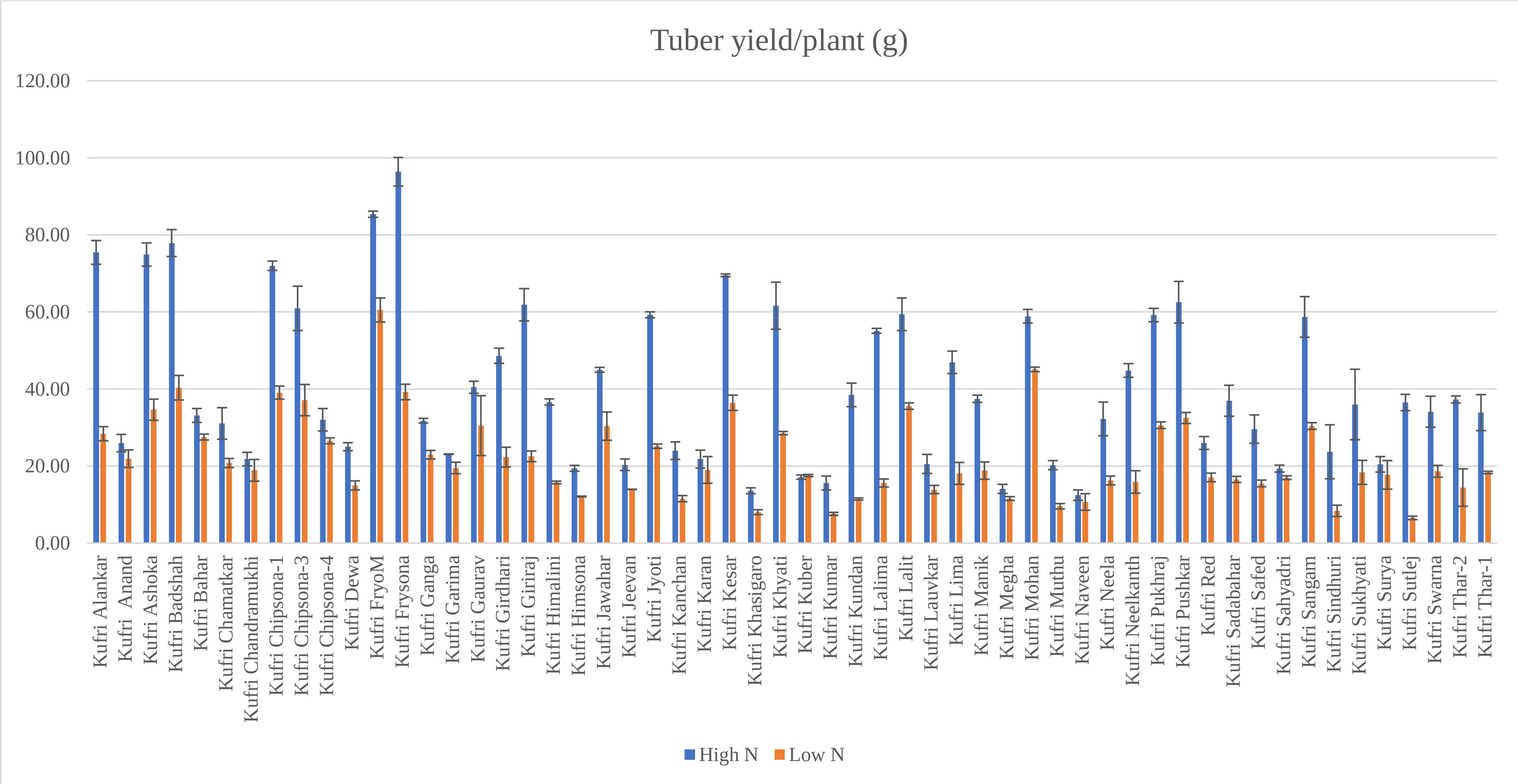

Fig 6.3.8. Graph depicting tuber yield per plant of 56 potato varieties under high & low N regimes.

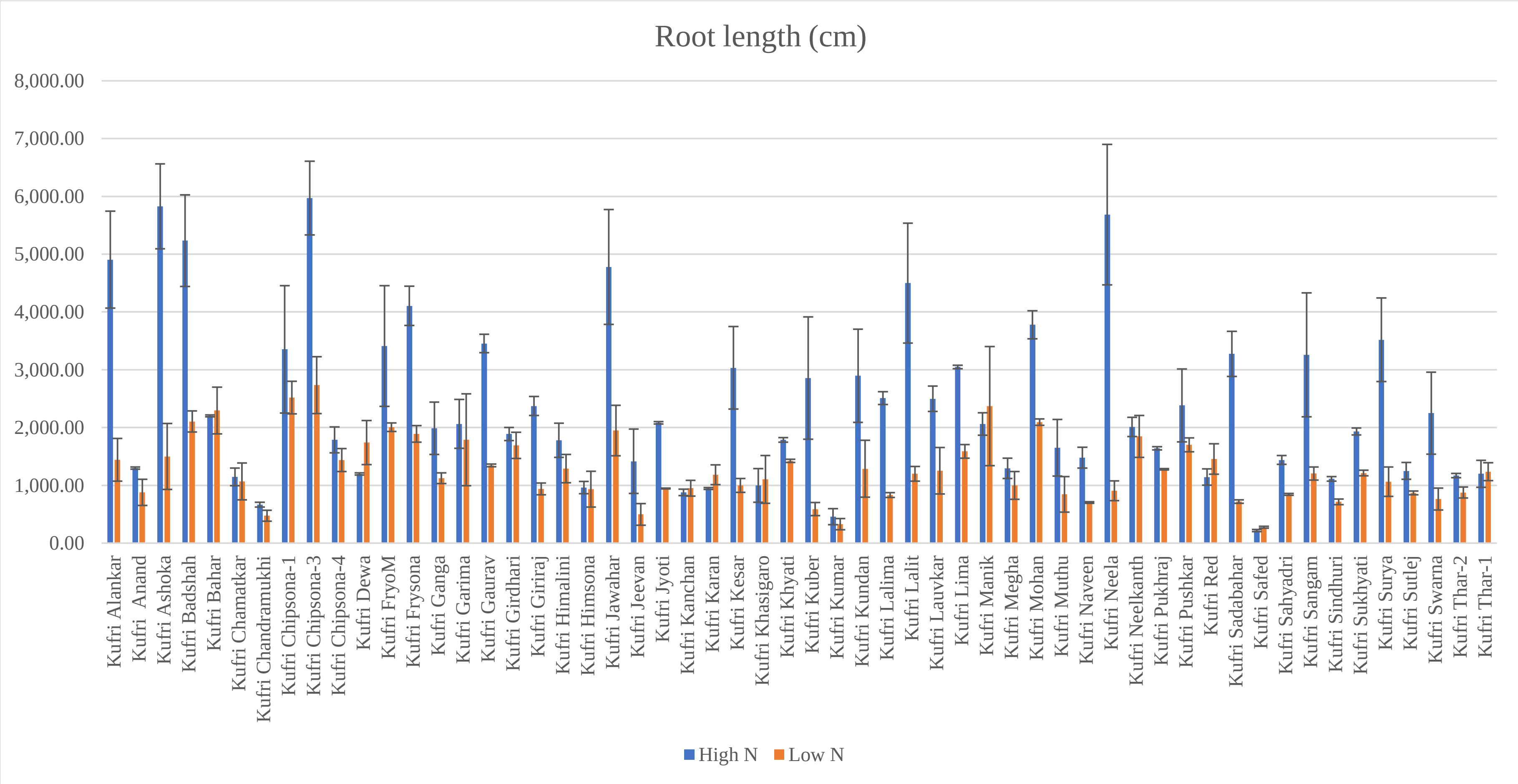

Fig 6.3.9. Graph depicting root length of 56 potato varieties under high & low N regimes.

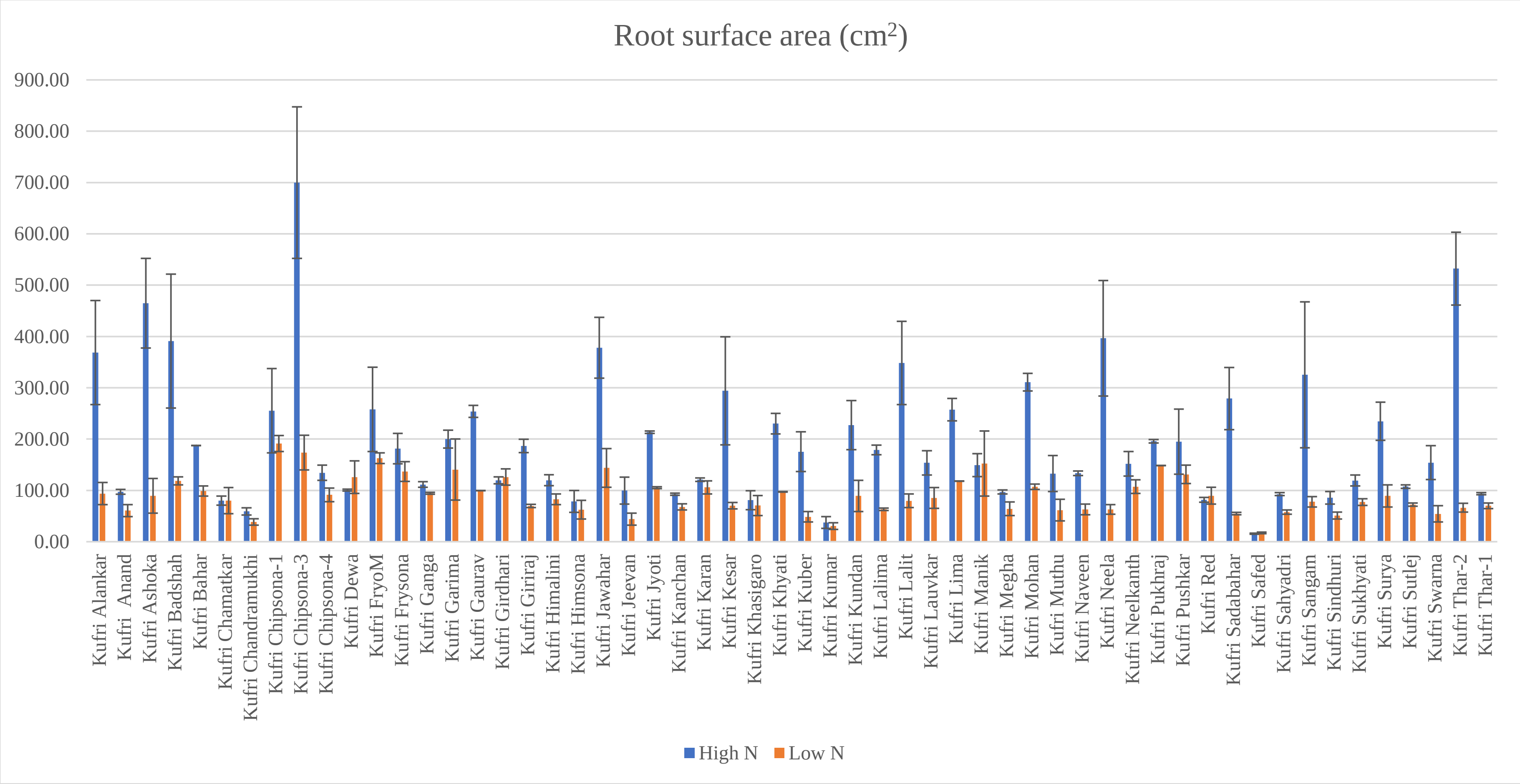

Fig 6.3.10. Graph depicting root surface area of 56 potato varieties under high & low N regimes.

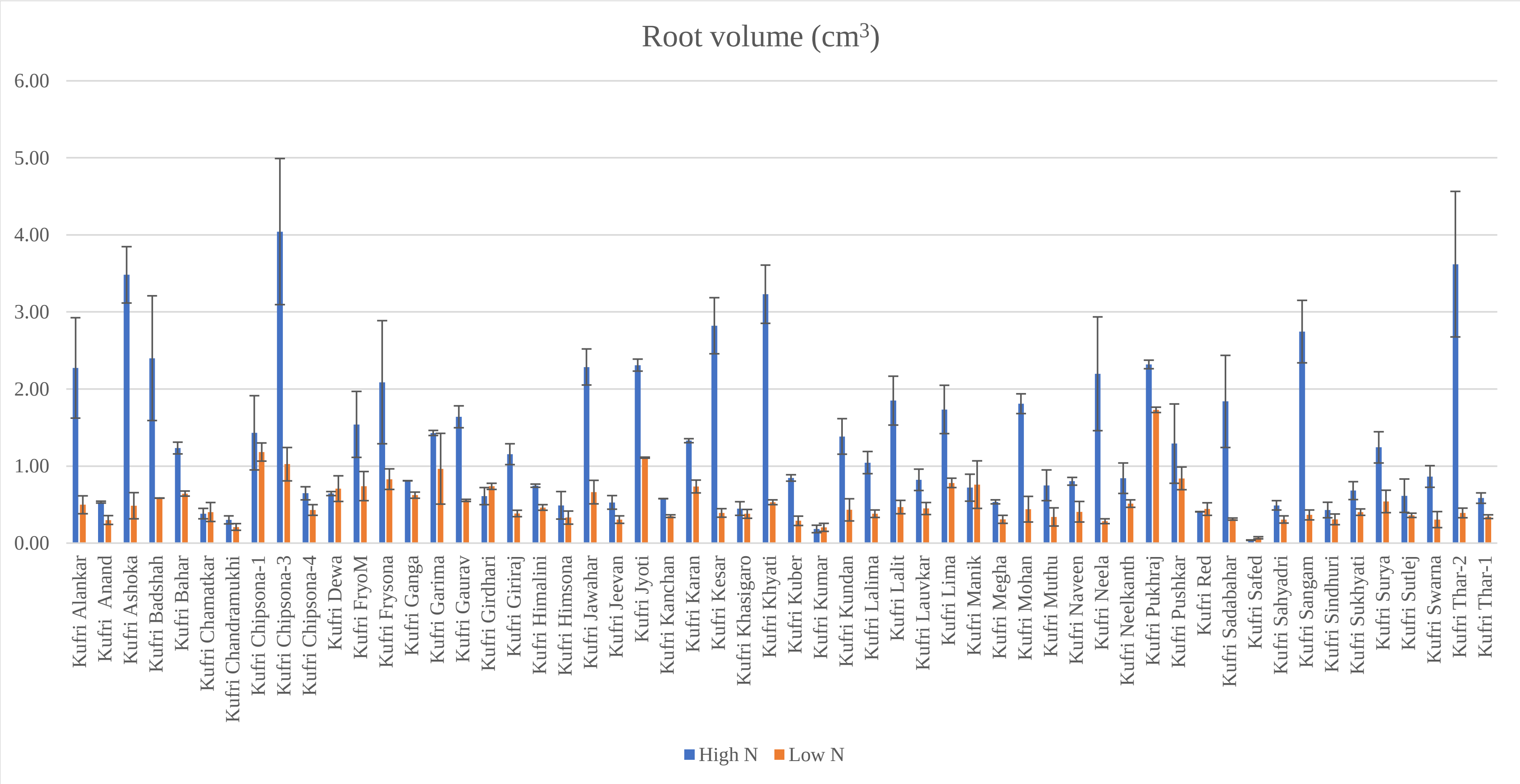

Fig 6.3.11. Graph depicting root volume of 56 potato varieties under high & low N regimes.

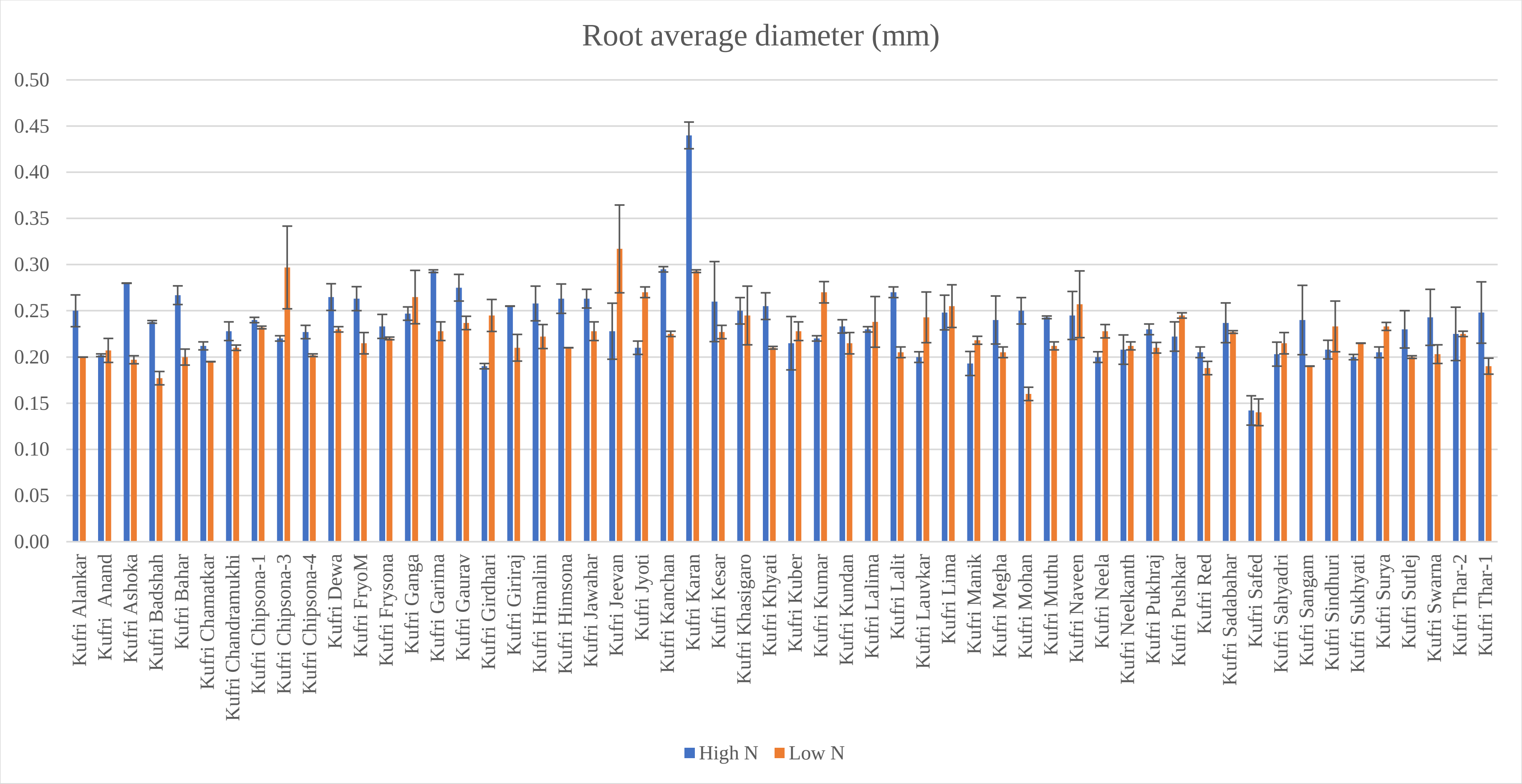

Fig 6.3.12. Graph depicting root average diameter of 56 potato varieties under high & low N regimes.

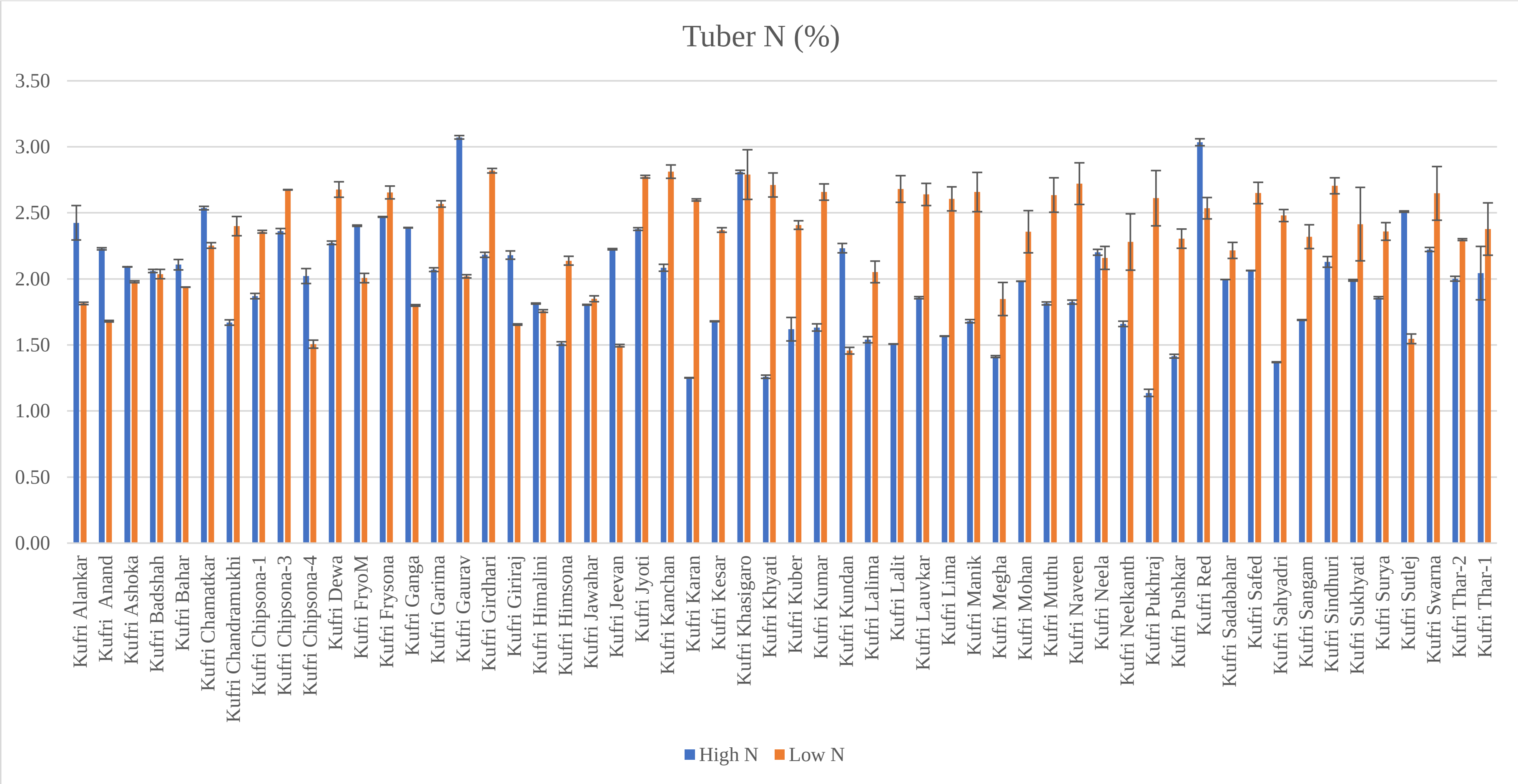

Fig 6.3.13. Graph depicting tuber nitrogen content of 56 potato varieties under high & low N regimes.

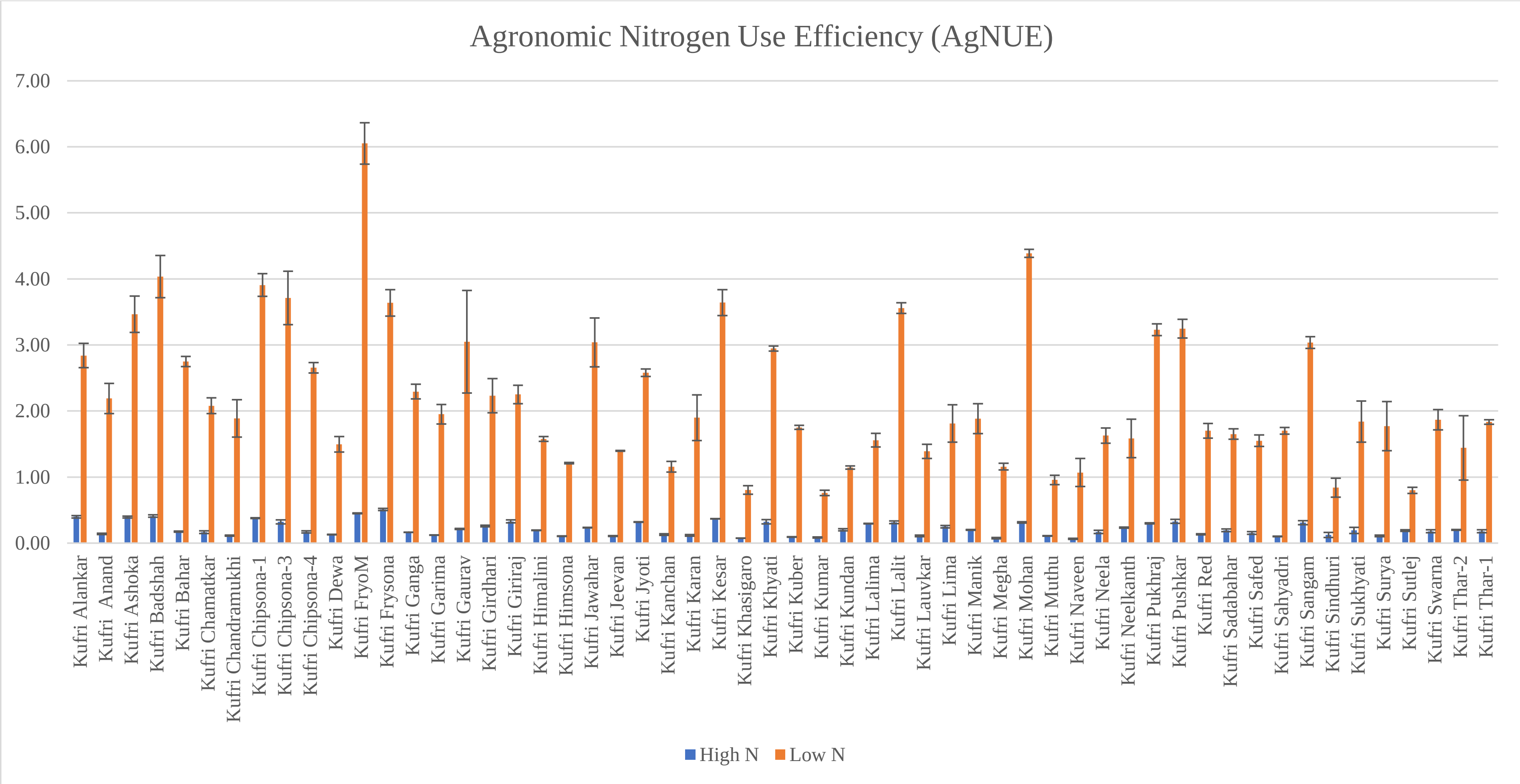

Fig 6.3.14. Graph depicting Agronomic Nitrogen Use Efficiency (AgNUE) of 56 potato varieties under high & low N regimes.

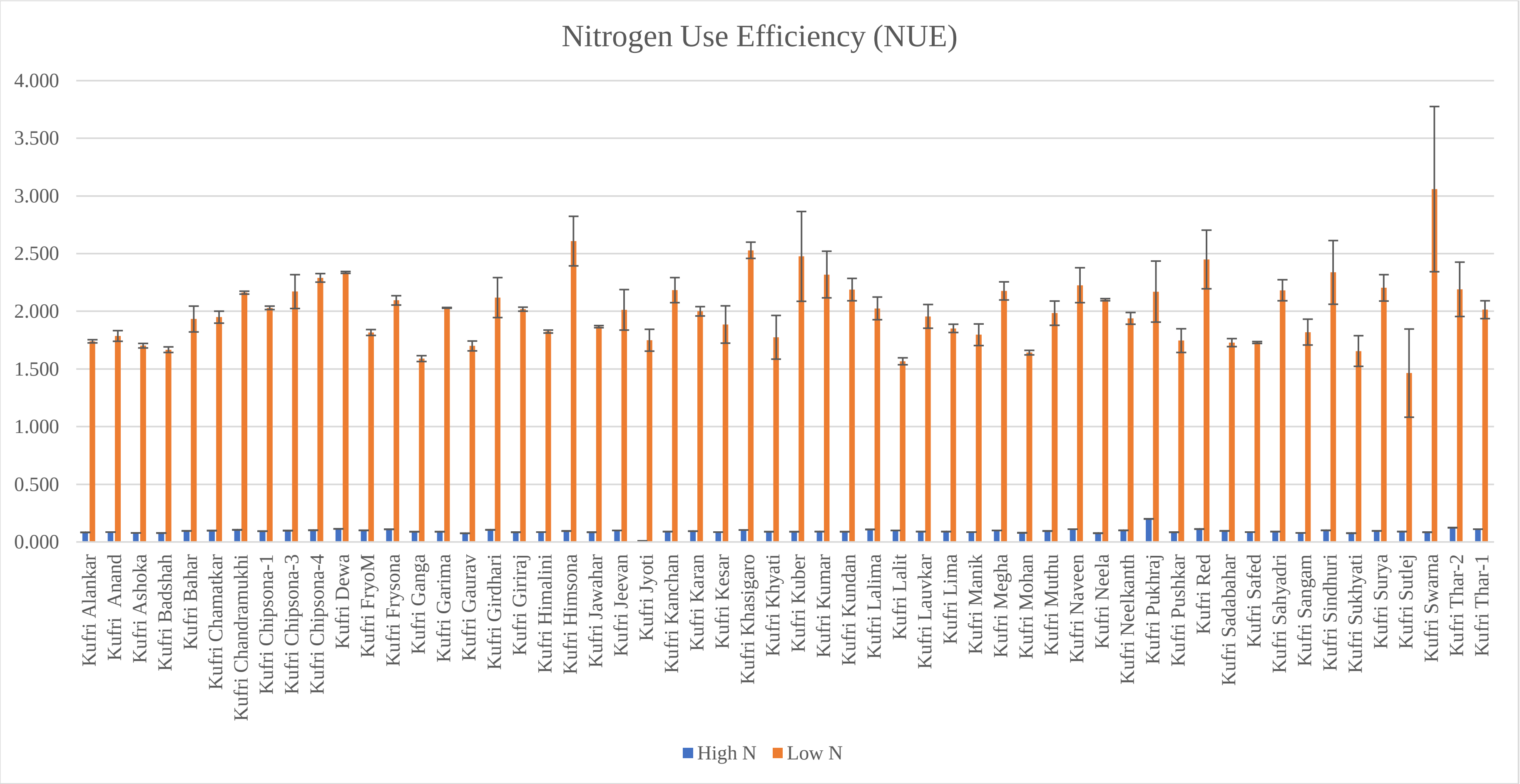

Fig 6.3.15. Graph depicting Nitrogen Use Efficiency (NUE) of 56 potato varieties under high & low N regimes.

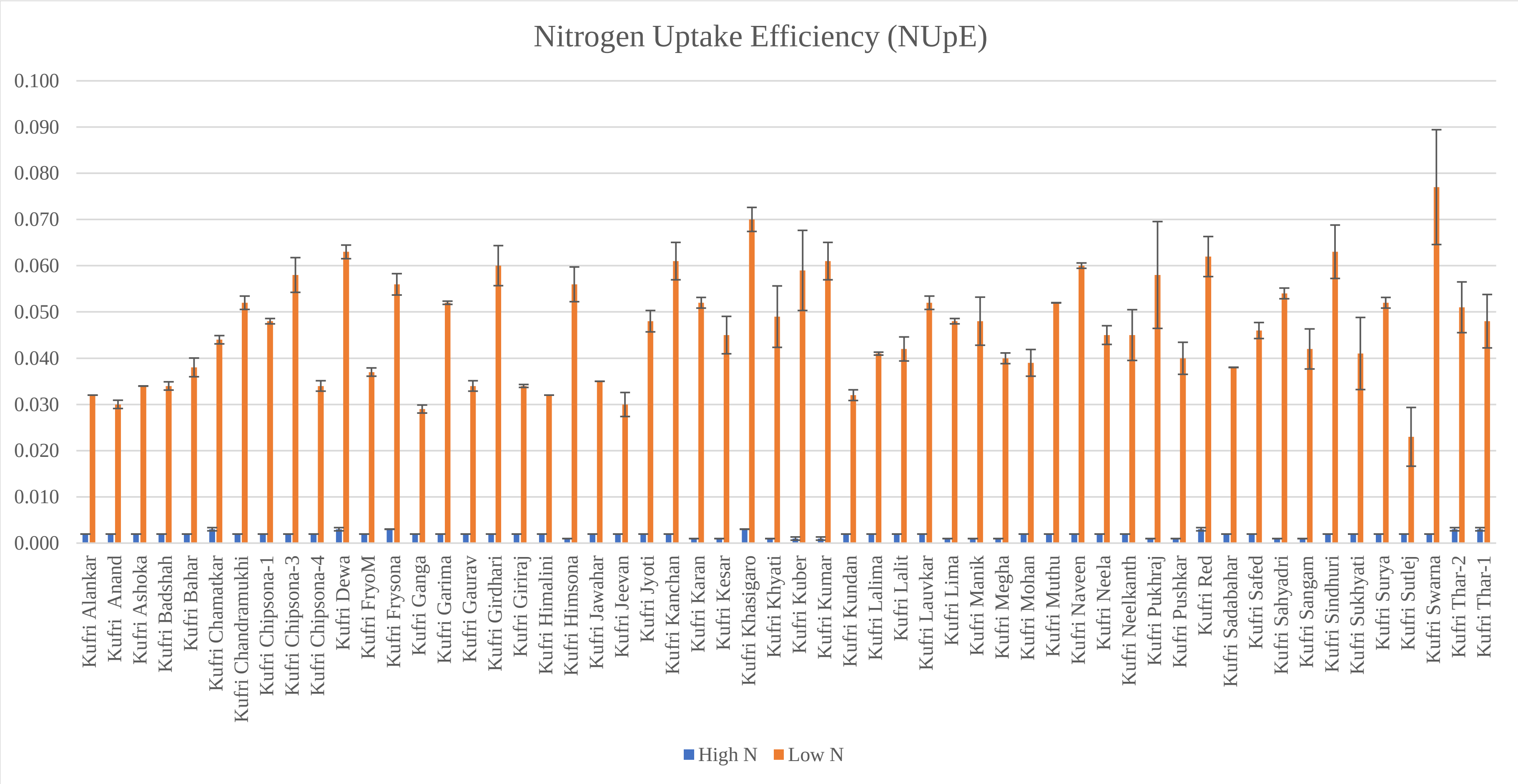

Fig 6.3.16. Graph depicting Nitrogen Uptake Efficiency (NUpE) of 56 potato varieties under high & low N regimes.

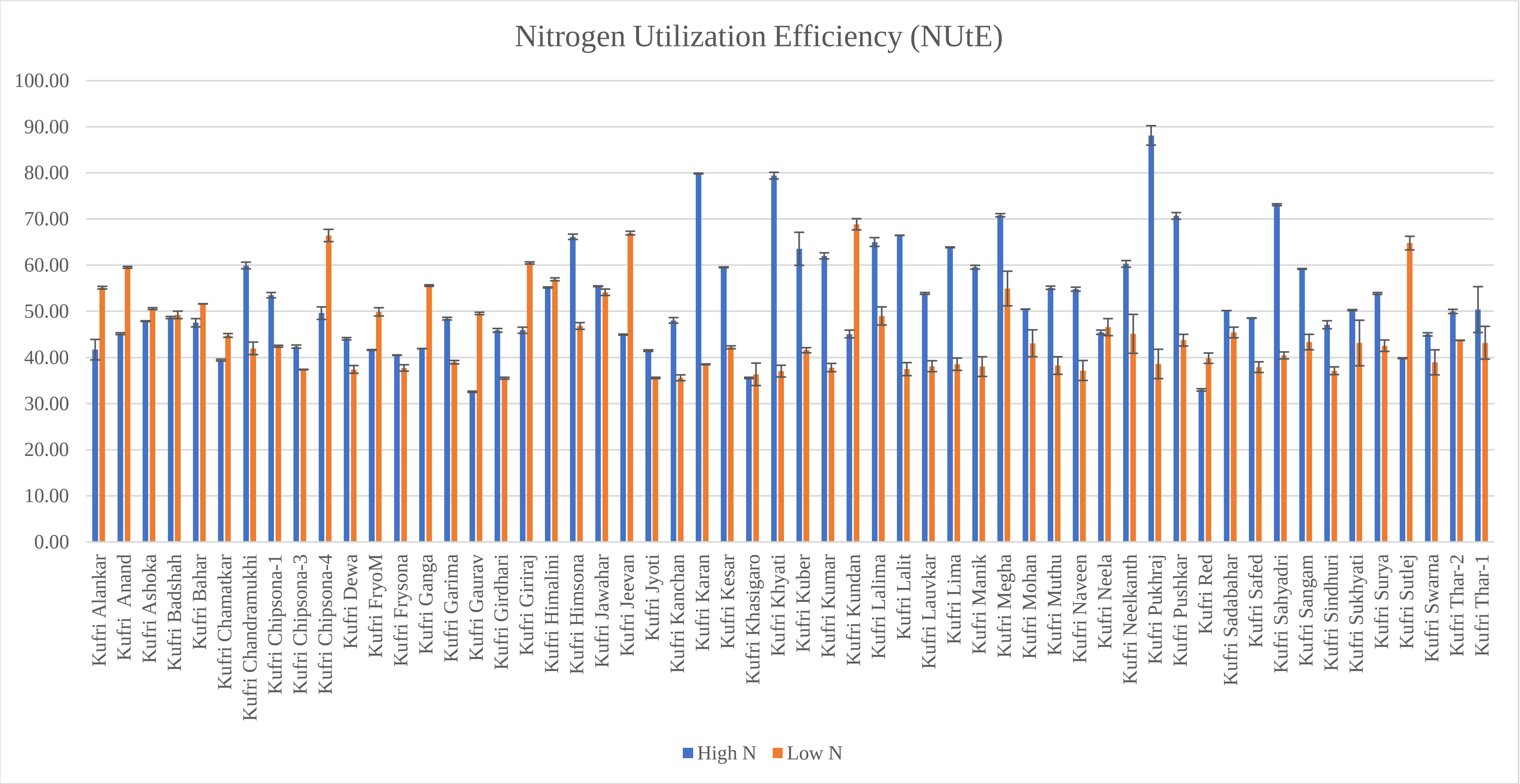

Fig 6.3.17. Graph depicting Nitrogen Utilization Efficiency (NUtE) of 56 potato varieties under high & low N regimes.

6.2 Transcriptome (RNA-seq) dynamics of potato varieties under different N regimes for tuber yield in aeroponics

6.2.1 Transcriptome sequencing (RNA-seq)

Based on the initial aeroponics study outcome and their popularity, Kufri Jyoti (N inefficient) and Kufri Pukhraj (N efficient) were selected for transcriptome sequencing. The Illumina platform was used to sequence RNA from tuber and leaf tissues, producing high-quality reads with a range of 4.28 to 5.46 Gb per sample. These readings showed a 72.40% to 78.20% similarity when mapped to the reference potato genome. Cufflinks was used to assemble transcriptome data, and Cuffdiff software was used to identify differentially expressed genes (DEGs). The tuber and leaf tissues of the Kufri Jyoti and Kufri Pukhraj cultivars were analyzed under high nitrogen (N) conditions versus low nitrogen (LN) controls. (Table 6.6). Statistical significance criteria ($p \leq 0.05$) were used to identify significant DEGs, with down-regulated genes showing a reduction of ≤ -2 Log2 FC and up-regulated genes showing an increase of ≥ 2 Log2 FC. The DEGs summary is provided in Table 6.7.

A total of 18485 DEGs were found in the tuber tissues of Kufri Jyoti when comparing high nitrogen (N) conditions to low N (control). Of these, 222 genes were down-regulated and 452 genes were significantly up-regulated ($p < 0.05$). When tubers from Kufri Pukhraj were analyzed under high and low N conditions, a total of 17344 DEGs were found, with 246 genes showing significant up-regulation and 336 showing down-regulation. Similarly, a total of 17990 DEGs were found in the leaf tissues of Kufri Jyoti when comparing high N to low N (control). Of these, 327 genes were down-regulated and 549 genes were significantly up-regulated ($p < 0.05$). A total of 17860 DEGs were found in Kufri Pukhraj leaves, of which 283 genes were down-regulated and 484 genes were significantly up-regulated. The top 20 up-regulated and down-regulated genes in both varieties under high N versus low N conditions are presented in Table 6.8 (leaf tissue) and Table 6.9 (tuber tissue).

Several genes were discovered to express themselves exclusively in the test or control samples. The Kufri Pukhraj potato cultivar's top 50 differentially expressed genes (DEGs) in high nitrogen (HN) conditions are shown in a heat map in Figures 6.5 (tuber) and 6.6 (leaf), compared to low nitrogen (LN) conditions (control). Furthermore, when comparing the tuber and leaf tissues of Kufri Pukhraj under HN and LN conditions, scatter plots and volcano plots demonstrate the markedly up-regulated and down-regulated DEGs. According to Figure 6.7, a Venn diagram study comparing Kufri Jyoti (HN) and Kufri Pukhraj (HN) in the tubers showed eight up-regulated and two down-regulated genes. With 45 up-regulated and 28 down-regulated genes between Kufri Jyoti (HN) and Kufri Pukhraj (HN), a greater number of shared genes were discovered in the leaf tissues (Figure 6.7). Two down-regulated genes and fourteen up-regulated genes were detected in both tuber and leaf tissues of Kufri Jyoti, according to another Venn diagram (Figure 6.8). Between the two tissues, Kufri Pukhraj showed five down-regulated genes and two up-regulated genes. Interestingly, osmotin genes were consistently up-regulated in both Kufri Pukhraj or Kufri Jyoti's tuber and leaf tissues, suggesting that they play an important part in plant stress response.

6.2.2 Identification of potential DEGs

In comparison to low nitrogen levels, a total of 20 genes were found to be either down-regulated (Log2 FC < -2.0; $p < 0.05$) or up-regulated (Log2 FC > 2.0; $p < 0.05$) under high nitrogen conditions. Table 6.8 (tuber) and Table 6.9 (leaf) tissues provide a summary of these genes. When comparing the tuber and leaf tissues of the two potato varieties grown in high nitrogen conditions to the control (low nitrogen), the analysis of differentially expressed genes (DEGs) revealed that genes linked to stress response, sugar metabolism, and transcription factors were significantly expressed.

For instance, a number of genes, such as sterol desaturase (Log_2FC 5.44), phosphoethanolamine N-methyltransferase (Log_2FC 4.64), and nitrate reductase (Log_2FC 4.2), were discovered to be up-regulated in the tuber tissues of the Kufri Jyoti variety (Table 6.8). In contrast, genes such as 20G-Fe(II) oxidoreductase (Log_2FC -3.64), 1-aminocyclopropane-1-carboxylate oxidase (Log_2FC -3.36), and xyloglucan endo-transglycosylase (Log_2FC -2.86) were down-regulated in Kufri Jyoti's tuber tissues. Similarly, aquaporin TIP1;3 (Log_2FC 4.24), multicystatin (Log_2FC 3.59), and trans-2-enoyl CoA reductase (Log_2FC 3.0) were among the most up-regulated genes found in the tuber tissues of the Kufri Pukhraj variety. Conversely, early nodulin (Log_2FC -6.61), hypoxia-induced protein (Log_2FC -5.37), and the conserved area containing the RING-H2 finger protein ATL2 B (Log_2FC -4.76) were among the genes that were down-regulated in the tubers of Kufri Pukhraj.

DEGs were identified in the leaves of both Kufri Jyoti and Kufri Pukhraj (Table

6.9). Numerous genes were identified to be up-regulated in Kufri Jyoti, such as endochitinase 4 (6.15), protein kinase (6.38), and multicystatin (7.93). On the other hand, the genes that were down-regulated in Kufri Jyoti's leaf tissues were proline oxidase/dehydrogenase 1 (-4.29), purine transporter (-5.60), and sodium/proline symporter (-5.90). Aquaporin TIP2;3 (5.34), xyloglucan endotransglucosylase/hydrolase 1 (5.77), and multicopper oxidase (5.83) were among the genes that were up-regulated in Kufri Pukhraj. Pectinesterase (-5.32), purine transporter (-5.29), and MYB transcription factor MYB139 (-5.02) were the genes that were down-regulated in Kufri Pukhraj.

This study also found genes that respond to nitrogen stress and nitrogen sufficiency in four different combinations of DEGs. In particular, it compared Kufri Pukhraj to Kufri Jyoti (control) in both tuber and leaf tissues under low and high nitrogen conditions (Tables 6.10 and 6.11). Among the genes that were up-regulated in Kufri Pukhraj as opposed to Kufri Jyoti (control) for tuber tissues under low nitrogen circumstances were sterol desaturase, miraculin, and cysteine protease inhibitor 1 (Table 6.10). In contrast, methylketone synthase Ib and acidic endochitinase were among the genes that were down-regulated. Comparing Kufri Pukhraj to Kufri Jyoti (control), the up-regulated genes for leaf tissues under low nitrogen conditions were pectinesterase and apyrase 3, while the down-regulated genes were zinc finger protein and phenylacetaldehyde synthase (Table 6.11). As seen in Tables 6.10 and 6.11, several genes also showed differential expression in high nitrogen conditions.

6.2.3 Gene Ontology (GO) characterization

Three classes of Gene Ontology (GO) terms—cellular component, molecular function, and biological process—were used to functionally annotate the differentially expressed genes (DEGs). In tuber and leaf tissues of both varieties, molecular function had the most associated genes (44096) among these categories, followed by biological process (36774) and cellular component (32736) (Table 6.12). We found 16207 cellular component terms, 18141 biological process phrases, and 21980 molecular function terms in the tubers. On the other hand, we found 16529 cellular component terms, 18633 biological process terms, and 22116 molecular function terms in the leaves. In both tissues, it was discovered that a number of GO keywords were significantly enriched among both up- and down-regulated DEGs. Cell, cell part, membrane, membrane part, binding, catalytic activity, metabolic process, and cellular process were among these terms (Fig. 6.9). Figure 6.10 displays the volcano plot and scatter plot illustrating the genes.

6.2.4 KEGG pathways analysis

Differentially expressed genes (DEGs) were classified into 24 KEGG functional pathways after being annotated. Only 21113 genes out of the 74268 total discovered genes were given KEGG annotations (Table 6.13 and Table 6.14). Signal transduction (2,475 genes), translation (1,942 genes), carbohydrate metabolism (1,906 genes), folding, sorting, and degradation (1,667 genes), transport and catabolism (1,511 genes), amino acid metabolism (1,260 genes), energy metabolism (1,180 genes), lipid metabolism (1,098 genes), and environmental adaptation (1,063 genes) were the main KEGG pathways that these genes represented in the tissues (Fig. 6.11). This highlights how important different gene networks are for nitrogen metabolism, especially the genes linked to signal transduction and carbohydrate metabolism, which are crucial for potato tuber growth and development.

6.2.5 Validation of selected candidate genes by real-time quantitative PCR (qRT-PCR) analysis

RT-qPCR analysis was used to validate eight chosen genes. The RING-H2 finger protein ATL2B (PGSC0003DMG400027871), aquaporin TIP1;3 (PGSC0003DMG400028182), 20G-Fe(II) oxidoreductase (PGSC0003DMG400030362), and nitrate reductase (PGSC0003DMG400030212) were among the genes found in tuber tissues. The following genes were analyzed in leaf tissues: xyloglucan endotransglucosylase/hydrolase 1 (PGSC0003DMG400024755), sodium/proline symporter (PGSC0003DMG400009706), multicystatin (PGSC0003DMG400005950), and a purine transporter (PGSC0003DMG400009706. Although there were minor differences in gene expression, the RT-qPCR gene expression impressions were in line with the transcriptome results (Table 6.15).

Table 6.6. RNA-seq data summary and reference mapping with the Potato genome

Sr. No.	Sample	Raw data	Reference mapping
Tuber			
1.	Kufri Jyoti (HN)-R1	5.32 Gb	75.60 %
2.	Kufri Jyoti (HN)-R2	4.28 Gb	72.40 %
3.	Kufri Jyoti (LN)-R1	5.46 Gb	72.80 %
4.	Kufri Jyoti (LN)-R2	5.13 Gb	74.50 %
5.	Kufri Pukhraj (HN)-R1	4.74 Gb	75.20 %
6.	Kufri Pukhraj (HN)-R2	5.10 Gb	72.80 %
7.	Kufri Pukhraj (LN)-R1	5.06 Gb	74.30 %
8.	Kufri Pukhraj (LN)-R2	5.20 Gb	73.50 %
Leaf			
9.	Kufri Jyoti (HN)-R1	4.35 Gb	74.60 %
10.	Kufri Jyoti (HN)-R2	4.65 Gb	78.20 %
11.	Kufri Jyoti (LN)-R1	4.36 Gb	75.90 %
12.	Kufri Jyoti (LN)-R2	5.10 Gb	72.60 %
13.	Kufri Pukhraj (HN)-R1	4.66 Gb	76.80 %
14.	Kufri Pukhraj (HN)-R2	4.80 Gb	75.10 %
15.	Kufri Pukhraj (LN)-R1	4.50 Gb	72.80 %
16.	Kufri Pukhraj (LN)-R2	5.20 Gb	76.50 %

HN: High N; LN: Low N

Table 6.7. DEGs summary

Combination#	Total DEGs	Significant DEGs ($p < 0.05$)			
		Up-regulated	Down-regulated	Exclusive (Control)	Exclusive (Treatment)
Tuber					
Kufri Jyoti	18485	452	222	143	497
Kufri Pukhraj	17344	246	336	173	127
Leaf					
Kufri Jyoti	17990	549	327	118	216
Kufri Pukhraj	17860	484	283	161	280

HN: High N, LN: Low N; DEGs analysis was performed in HN versus LN (control) of the same variety.

Table 6.8. Selected top differentially expressed genes in tuber tissues of potato varieties under high N versus low N (control) under aeroponics

Sr. No.	Gene ID	Gene expression (Log_2 FC)	P value	Gene description
i) Kufri Jyoti (HN vs. LN)				
Up-regulated				
1	PGSC0003DMG400028022	5.445	0.002	Sterol desaturase
2	PGSC0003DMG400014459	4.640	0.024	Phosphoethanolamine N-methyltransferase
3	PGSC0003DMG400030212	4.252	0.020	Nitrate reductase
4	PGSC0003DMG400028305	4.210	0.032	Heat shock protein binding protein
5	PGSC0003DMG400013547	4.163	0.000	Sucrose synthase
6	PGSC0003DMG400028396	3.946	0.017	Phosphate transporter PHO1 homolog 10
7	PGSC0003DMG400015229	3.672	0.000	BTB/POZ domain-containing protein
8	PGSC0003DMG402031759	3.638	0.015	Phospholipase A1
9	PGSC0003DMG400004378	3.428	0.046	GDSL esterase/lipase
10	PGSC0003DMG400012479	3.306	0.043	Nitrate transporter
Down-regulated				
1	PGSC0003DMG400018505	-3.688	0.038	DAD1
2	PGSC0003DMG400030362	-3.645	0.014	20G-Fe(II) oxidoreductase

3	PGSC0003DMG401026923	-3.369	0.017	1-aminocyclopropane-1-carboxylate oxidase
4	PGSC0003DMG400015129	-3.060	0.001	Defensin protein
5	PGSC0003DMG400021877	-2.860	0.000	Xyloglucan endo-transglycosylase
6	PGSC0003DMG400004109	-2.776	0.000	Xyloglucan endotransglycosylase hydrolase
7	PGSC0003DMG400008000	-2.660	0.001	L-asparaginase
8	PGSC0003DMG400026461	-2.470	0.011	AP2/ERF domain-containing transcription factor
9	PGSC0003DMG400007994	-2.270	0.014	Tuber-specific and sucrose-responsive element binding factor
10	PGSC0003DMG400001418	-2.223	0.003	Transcription factor style2.1
ii) Kufri Pukhraj (HN vs. LN)				
Up-regulated				
1	PGSC0003DMG400028182	4.240	0.015	Aquaporin TIP1;3
2	PGSC0003DMG400018286	3.767	0.020	Vetispiradiene synthase
3	PGSC0003DMG400026899	3.591	0.001	Multicystatin
4	PGSC0003DMG400029260	3.008	0.013	Trans-2-enoyl CoA reductase
5	PGSC0003DMG400020388	2.967	0.029	Cationic peroxidase 1
6	PGSC0003DMG400003044	2.702	0.000	Osmotin
7	PGSC0003DMG400013411	2.482	0.000	Chlorophyll a-b binding protein 3C, chloroplastic

8	PGSC0003DMG400023366	2.457	0.000	Quinonprotein alcohol dehydrogenase
9	PGSC0003DMG400003512	2.431	0.034	Laccase
10	PGSC0003DMG400016573	2.343	0.002	Glutaredoxin
Down-regulated				
1	PGSC0003DMG400020681	-6.619	0.000	Early nodulin
2	PGSC0003DMG400027976	-5.376	0.003	Hypoxia induced protein conserved region containing protein
3	PGSC0003DMG400027871	-4.769	0.004	RING-H2 finger protein ATL2B
4	PGSC0003DMG400002804	-4.357	0.000	USP
5	PGSC0003DMG400039214	-4.030	0.002	Arachidonic acid-induced DEA1
6	PGSC0003DMG400008000	-4.020	0.017	L-asparaginase
7	PGSC0003DMG400024754	-3.740	0.000	Respiratory burst oxidase homolog protein B
8	PGSC0003DMG400030212	-3.538	0.002	Nitrate reductase
9	PGSC0003DMG400006678	-3.256	0.000	Aspartate aminotransferase
10	PGSC0003DMG400030362	-3.004	0.002	20G-Fe(II) oxidoreductase

DEGs analysis was performed in sample of high N (HN) versus low N (LN, control) of the same variety.

Table 6.9. Selected top differentially expressed genes in leaf tissues of potato varieties under high N versus low N (control) under aeroponics.

Sr. No.	Gene ID	Gene expression (Log$_2$ FC)	P value	Gene description
i) Kufri Jyoti (HN vs. LN)				
Up-regulated				
1	PGSC0003DMG400005950	7.930	0.000	Multicystatin
2	PGSC0003DMG400018644	6.382	0.020	Protein kinase
3	PGSC0003DMG400026855	6.155	0.009	Endochitinase 4
4	PGSC0003DMG400009513	6.047	0.007	Aspartic protease inhibitor 5
5	PGSC0003DMG400019517	5.510	0.000	Chitin-binding lectin 1
6	PGSC0003DMG400013537	5.451	0.016	Proline-rich protein
7	PGSC0003DMG403020240	4.263	0.006	Glycerophosphodiester phosphodiesterase
8	PGSC0003DMG400004170	4.126	0.007	Asparagine synthetase
9	PGSC0003DMG400018286	3.966	0.003	Vetispiradiene synthase
10	PGSC0003DMG400030784	3.869	0.000	Glutaredoxin family protein
Down-regulated				
1	PGSC0003DMG401007615	-5.909	0.000	Sodium/proline symporter
2	PGSC0003DMG400009706	-5.609	0.003	Purine transporter

3	PGSC0003DMG400007683	-4.903	0.006	Sulfate/bicarbonate/oxalate exchanger and transporter sat-1
4	PGSC0003DMG400010050	-4.293	0.000	Proline oxidase/dehydrogenase 1
5	PGSC0003DMG400018129	-3.792	0.031	High-affinity nitrate transport system component
6	PGSC0003DMG400008000	-3.574	0.000	L-asparaginase
7	PGSC0003DMG402010883	-3.556	0.010	MYB transcription factor MYB139
8	PGSC0003DMG400013443	-3.310	0.001	Acyltransferase
9	PGSC0003DMG400009570	-3.284	0.003	MYB transcription factor
10	PGSC0003DMG400009705	-3.002	0.000	Purine transporter
ii) Kufri Pukhraj (HN vs. LN)				
Up-regulated				
1	PGSC0003DMG401016475	5.837	0.043	Multicopper oxidase
2	PGSC0003DMG400024755	5.776	0.000	Xyloglucan endotransglucosylase/ hydrolase 1
3	PGSC0003DMG400009514	5.711	0.036	Kunitz-type protease inhibitor
4	PGSC0003DMG400009513	5.642	0.013	Aspartic protease inhibitor 5
5	PGSC0003DMG400026463	5.345	0.000	Aquaporin TIP2;3
6	PGSC0003DMG400003040	4.653	0.002	Osmotin
7	PGSC0003DMG400023620	4.314	0.000	Glutamine synthetase
8	PGSC0003DMG400029201	4.186	0.023	Sesquiterpene synthase 2

9	PGSC0003DMG400010765	4.153	0.023	Glutaredoxin
10	PGSC0003DMG400013815	2.984	0.004	Nitrate transporter
Down-regulated				
1	PGSC0003DMG400025967	-5.322	0.001	Pectinesterase
2	PGSC0003DMG400009706	-5.291	0.026	Purine transporter
3	PGSC0003DMG400000110	-5.061	0.008	Wax synthase
4	PGSC0003DMG402010883	-5.022	0.022	MYB transcription factor MYB139
5	PGSC0003DMG400012020	-4.335	0.002	Pectin methlyesterase inhibitor protein 1
6	PGSC0003DMG400000184	-4.151	0.001	Ferric-chelate reductase
7	PGSC0003DMG400019671	-4.134	0.023	Glutaredoxin
8	PGSC0003DMG400031360	-4.083	0.028	UDP-glucoronosyl/UDP-glucosyl transferase family protein
9	PGSC0003DMG400019293	-3.774	0.005	NAC domain-containing protein
10	PGSC0003DMG400026148	-3.728	0.005	USP family protein

DEGs analysis was performed in sample of HN versus low N (LN, control) of the same variety.

Table 6.10. Selected top differentially expressed genes in tuber tissues of potato varieties Kufri Pukhraj versus Kufri Jyoti (control) under different N regimes in aeroponics

Sr. No.	Gene ID	Gene expression (Log_2 FC)	P value	Gene description
i) Low N (Kufri Pukhraj vs. Kufri Jyoti)				
Up-regulated				
1.	PGSC0003DMG400010139	12.223	0.006	Cysteine protease inhibitor 1
2.	PGSC0003DMG400010170	10.784	0.008	Miraculin
3.	PGSC0003DMG400028022	6.624	0.006	Sterol desaturase
4.	PGSC0003DMG400010146	6.595	0.000	Kunitz-type tuber invertase inhibitor
5.	PGSC0003DMG400010169	6.303	0.000	Beta-carotene hydroxylase
6.	PGSC0003DMG400010143	6.152	0.000	Cysteine protease inhibitor 1
7.	PGSC0003DMG401021841	5.841	0.038	Replication factor A
8.	PGSC0003DMG401001552	5.463	0.032	3-isopropylmalate dehydratase
9.	PGSC0003DMG400008850	4.839	0.000	Short-chain dehydrogenase
10.	PGSC0003DMG400012032	4.699	0.009	Gamma-gliadin
Up-regulated				
1.	PGSC0003DMG400004599	-6.352	0.002	Gene of unknown function

2.	PGSC0003DMG400022355	-5.393	0.034	Gene of unknown function
3.	PGSC0003DMG400025912	-5.169	0.005	Methylketone synthase Ib
4.	PGSC0003DMG400006247	-4.990	0.014	Conserved gene of unknown function
5.	PGSC0003DMG400033882	-4.617	0.009	Acidic endochitinase
6.	PGSC0003DMG400001418	-4.415	0.012	Transcription factor style2.1
7.	PGSC0003DMG401025826	-4.397	0.043	3-hydroxyisobutyryl-CoA hydrolase 1
8.	PGSC0003DMG401029345	-4.208	0.034	Isoform 2 of TMV resistance protein N
9.	PGSC0003DMG400029510	-4.180	0.013	ZFP4 (ZINC FINGER PROTEIN 4)
10.	PGSC0003DMG402005859	-4.084	0.012	Conserved gene of unknown function
ii) High N (Kufri Pukhraj vs. Kufri Jyoti)				
Up-regulated				
1.	PGSC0003DMG400010139	8.934	0.000	Cysteine protease inhibitor 1
2.	PGSC0003DMG400010170	7.260	0.000	Miraculin
3.	PGSC0003DMG400010169	6.688	0.001	Beta-carotene hydroxylase
4.	PGSC0003DMG401021841	5.840	0.040	Replication factor A
5.	PGSC0003DMG400005950	5.445	0.000	Multicystatin
6.	PGSC0003DMG400008850	5.249	0.000	Short-chain dehydrogenase
7.	PGSC0003DMG400025168	4.336	0.000	Lipid binding protein

8.	PGSC0003DMG400006800	4.241	0.003	NBS-LRR protein
9.	PGSC0003DMG400016867	4.109	0.016	Chalcone synthase J
10.	PGSC0003DMG400015129	4.043	0.000	Defensin protein
11.	PGSC0003DMG401005482	4.039	0.002	E2F4,5
Down-regulated				
1.	PGSC0003DMG400010145	-5.994	0.001	Cysteine protease inhibitor 9
2.	PGSC0003DMG400005683	-5.822	0.019	Gene of unknown function
3.	PGSC0003DMG400026617	-5.386	0.048	Methylketone synthase Ib
4.	PGSC0003DMG402005859	-4.855	0.014	Conserved gene of unknown function
5.	PGSC0003DMG400030212	-4.643	0.008	Nitrate reductase
6.	PGSC0003DMG400004599	-4.320	0.000	Gene of unknown function
7.	PGSC0003DMG400000816	-4.147	0.009	Tospovirus resistance protein A
8.	PGSC0003DMG400017091	-4.055	0.000	Patatin-01
9.	PGSC0003DMG404025785	-4.038	0.037	Dynamin
10.	PGSC0003DMG400003040	-4.025	0.025	Osmotin

DEGs analysis was performed in Kufri Pukhraj versus Kufri Jyoti (control) under low N and high N.

Table 6.11. Selected top differentially expressed genes in leaf tissues of potato varieties Kufri Pukhraj versus Kufri Jyoti (control) under different N regimes in aeroponics

Sr. No.	Gene ID	Gene expression (Log_2 FC)	P value	Gene description
i) Low N (Kufri Pukhraj vs. Kufri Jyoti)				
Up-regulated				
1.	PGSC0003DMG400002261	5.448	0.005	Conserved gene of unknown function
2.	PGSC0003DMG400025967	5.338	0.000	Pectinesterase
3.	PGSC0003DMG400007335	5.041	0.001	Apyrase 3
4.	PGSC0003DMG401021841	4.781	0.009	Replication factor A
5.	PGSC0003DMG400000292	4.592	0.000	Conserved gene of unknown function
6.	PGSC0003DMG400007385	4.568	0.003	CC-NB-LRR protein
7.	PGSC0003DMG402018893	4.474	0.003	Strictosidine synthase
8.	PGSC0003DMG400009931	4.216	0.036	Zinc-binding family protein
9.	PGSC0003DMG400015225	4.086	0.005	Transposase
10.	PGSC0003DMG401015362	3.926	0.000	ATORC3/ORC3
Down-regulated				
1.	PGSC0003DMG400003865	-4.864	0.017	Conserved gene of unknown function

2.	PGSC0003DMG400028701	-4.812	0.001	Zinc finger protein
3.	PGSC0003DMG400024278	-4.411	0.002	Phenylacetaldehyde synthase
4.	PGSC0003DMG400029635	-4.255	0.005	Histidine-rich glycoprotein
5.	PGSC0003DMG400032534	-4.234	0.000	Early nodulin 75 protein
6.	PGSC0003DMG400006247	-4.122	0.000	Conserved gene of unknown function
7.	PGSC0003DMG400002732	-4.106	0.025	VQ motif-containing protein
8.	PGSC0003DMG400004599	-3.971	0.000	Gene of unknown function
9.	PGSC0003DMG400025079	-3.855	0.029	Interferon-induced GTP-binding protein mx
10.	PGSC0003DMG400004259	-3.701	0.029	Thaumatin
ii) High N (Kufri Pukhraj vs. Kufri Jyoti)				
Up-regulated				
1.	PGSC0003DMG400020660	5.445	0.047	Protein kinase domain containing protein
2.	PGSC0003DMG400007385	5.080	0.023	CC-NB-LRR protein
3.	PGSC0003DMG400016013	4.840	0.020	Cytochrome P450
4.	PGSC0003DMG402000594	4.707	0.003	Flavonol synthase/flavanone 3-hydroxylase
5.	PGSC0003DMG400029623	4.341	0.015	Salicylic acid/benzoic acid carboxyl methyltransferase
6.	PGSC0003DMG400018924	4.007	0.027	Polyphenol oxidase
7.	PGSC0003DMG400007335	3.961	0.000	Apyrase 3

8.	PGSC0003DMG400007765	3.942	0.027	Sn-1 protein
9.	PGSC0003DMG400011601	3.917	0.005	2,4-dienoyl-CoA reductase
10.	PGSC0003DMG400029503	3.822	0.002	ETAG-A3
Down-regulated				
1.	PGSC0003DMG400006247	-5.850	0.002	Conserved gene of unknown function
2.	PGSC0003DMG400030842	-5.484	0.022	PTP-1
3.	PGSC0003DMG400023514	-5.432	0.039	Conserved gene of unknown function
4.	PGSC0003DMG400004599	-4.910	0.001	Gene of unknown function
5.	PGSC0003DMG400019517	-4.671	0.000	Chitin-binding lectin 1
6.	PGSC0003DMG400029085	-4.452	0.000	Mta/sah nucleosidase
7.	PGSC0003DMG400018012	-4.335	0.003	Conserved gene of unknown function
8.	PGSC0003DMG400000110	-4.285	0.016	Wax synthase
9.	PGSC0003DMG400011346	-4.251	0.023	Flowering promoting factor-like 1
10.	PGSC0003DMG400030820	-4.097	0.001	Gene of unknown function

DEGs analysis was performed in Kufri Pukhraj versus Kufri Jyoti (control) under low N and high N.

Table 6.12. GO annotation summary

Combination#	Description	Biological Process	Cellular Component	Molecular Function
Tuber				
Kufri Jyoti	Down-regulated	108	108	135
	Exclusive control	170	134	187
	Exclusive treated	31	40	45
	Expressed both	8818	7890	10759
	Up-regulated	281	209	306
Kufri Pukhraj	Down-regulated	184	156	203
	Exclusive control	37	32	36
	Exclusive treated	57	44	55
	Expressed both	8320	7499	10100
	Up-regulated	135	95	154
Sub-total (Tuber)		**18141**	**16207**	**21980**
Leaf				
Kufri Jyoti	Down-regulated	189	147	226
	Exclusive control	72	62	81
	Exclusive treated	20	30	27
	Expressed both	8698	7760	10390
	Up-regulated	374	292	380
Kufri Pukhraj	Down-regulated	157	125	187
	Exclusive control	115	90	112
	Exclusive treated	46	32	57
	Expressed both	8669	7747	10334
	Up-regulated	293	244	322
Sub-total (Leaf)		**18633**	**16529**	**22116**
Total (Tuber + Leaf)		**36774**	**32736**	**44096**

HN: High N, LN: Low N;

DEGs analysis was performed in HN versus LN (control) of the same variety.

Table 6.13. KEGG Annotation Statistics

Sample	Identified gene counts	KEGG Annotated gene counts
Tuber		
Kufri Jyoti	19345	5397
Kufri Pukhraj	17863	5126
Leaf		
Kufri Jyoti	18538	5278
Kufri Pukhraj	18522	5312
Total	**74268**	**21113**

HN: High N, LN: Low N;

DEGs analysis was performed in HN versus LN (control) of the same variety.

Table 6.14. KEGG Pathway classification

KEGG pathways	Tuber		Leaf	
	Kufri Jyoti	Kufri Pukhraj	Kufri Jyoti	Kufri Pukhraj
Metabolism				
Carbohydrate metabolism	486	458	481	481
Energy metabolism	291	282	303	304
Lipid metabolism	282	264	273	279
Nucleotide metabolism	95	95	93	94
Amino acid metabolism	321	306	318	315
Metabolism of other amino acids	150	134	142	140
Glycan biosynthesis and metabolism	130	123	130	128
Metabolism of cofactors and vitamins	213	201	215	218
Metabolism of terpenoids and polyketides	155	145	146	152
Biosynthesis of other secondary metabolites	204	175	166	166
Xenobiotics biodegradation and metabolism	84	70	72	73

KEGG pathways	Tuber		Leaf	
	Kufri Jyoti	Kufri Pukhraj	Kufri Jyoti	Kufri Pukhraj
Genetic Information Processing				
Transcription	216	214	210	212
Translation	494	482	485	481
Folding, sorting and degradation	426	418	410	413
Replication and repair	120	117	105	113
Environmental Information Processing				
Membrane transport	28	27	25	26
Signal transduction	631	586	627	631
Signaling molecules and interaction	2	2	2	2
Cellular Processes				
Transport and catabolism	379	370	382	380
Cell growth and death	269	259	266	271
Cellular community – eukaryotes	62	60	54	65
Cellular community - prokaryotes	50	46	50	53
Cell motility	41	40	43	42
Organismal Systems				
Environmental adaptation	268	252	270	273

HN: High N, LN: Low N;

DEGs analysis was performed in HN versus LN (control) of the same variety.

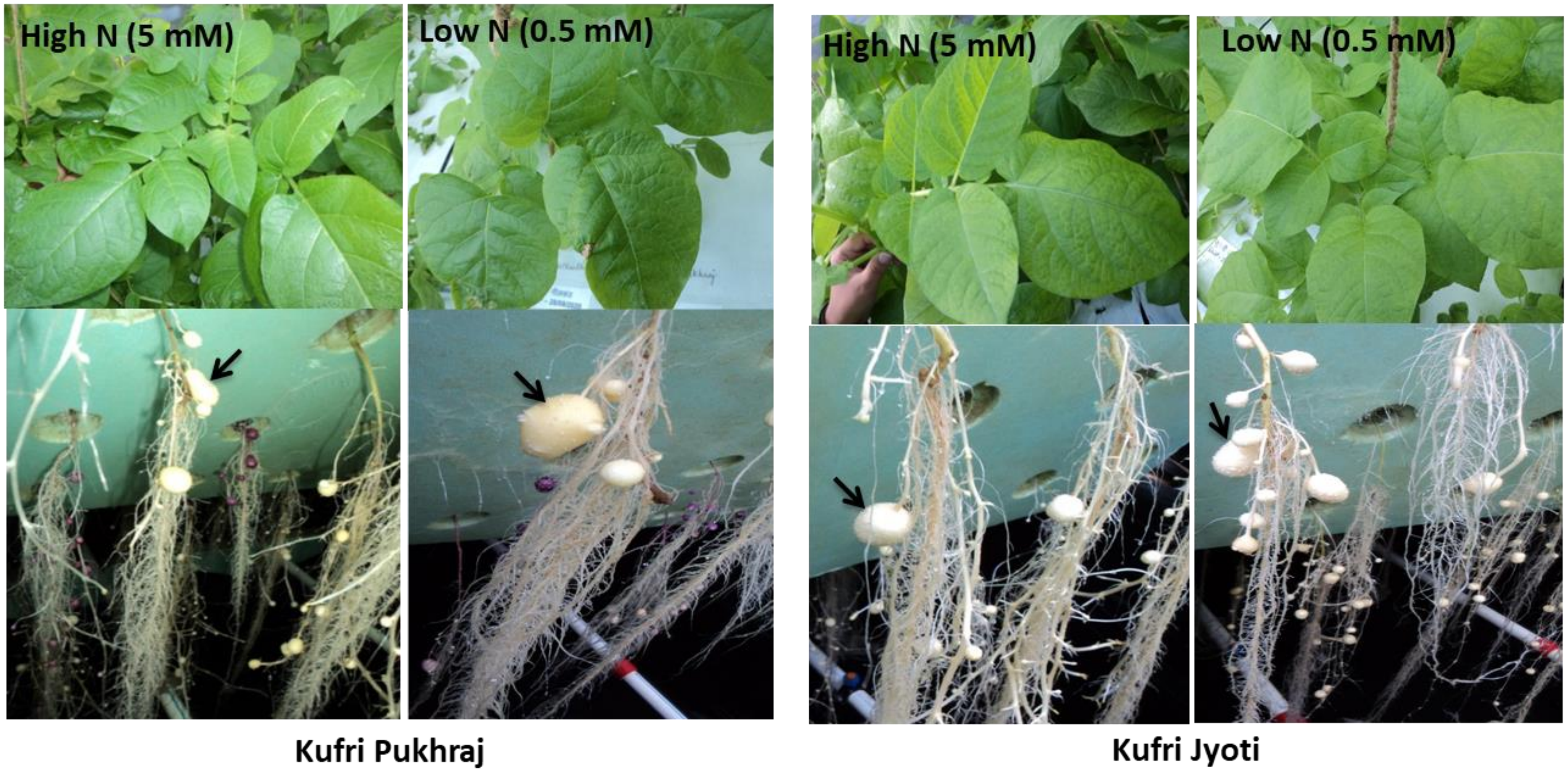

Fig. 6.4. Phenotypic performance of potato varieties Kufri Jyoti and Kufri Pukhraj under high N (5 mM) and low N (0.5 mM) regimes in aeroponics conditions. The arrow indicates tuber formation in these potato varieties.

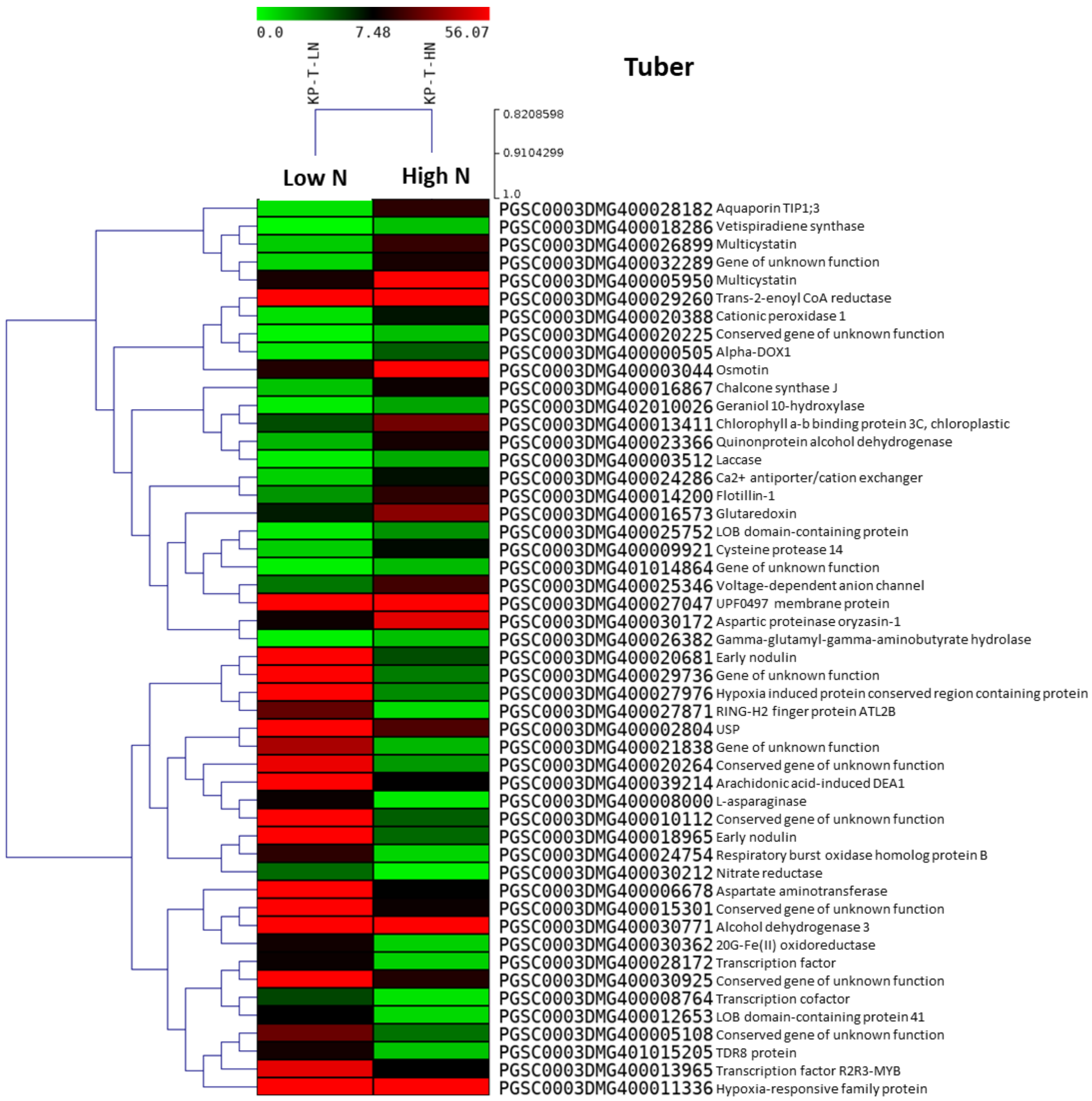

Fig.6.5. Heat maps of the top 50 differentially expressed genes ($p < 0.05$) in tuber tissues of potato variety Kufri Pukhraj under high N (low N (control)) by RNA-seq. In a heat map, each horizontal line refers to a gene. Relatively up-regulated genes are shown in red colour, whereas down-regulated genes are shown in green colour.

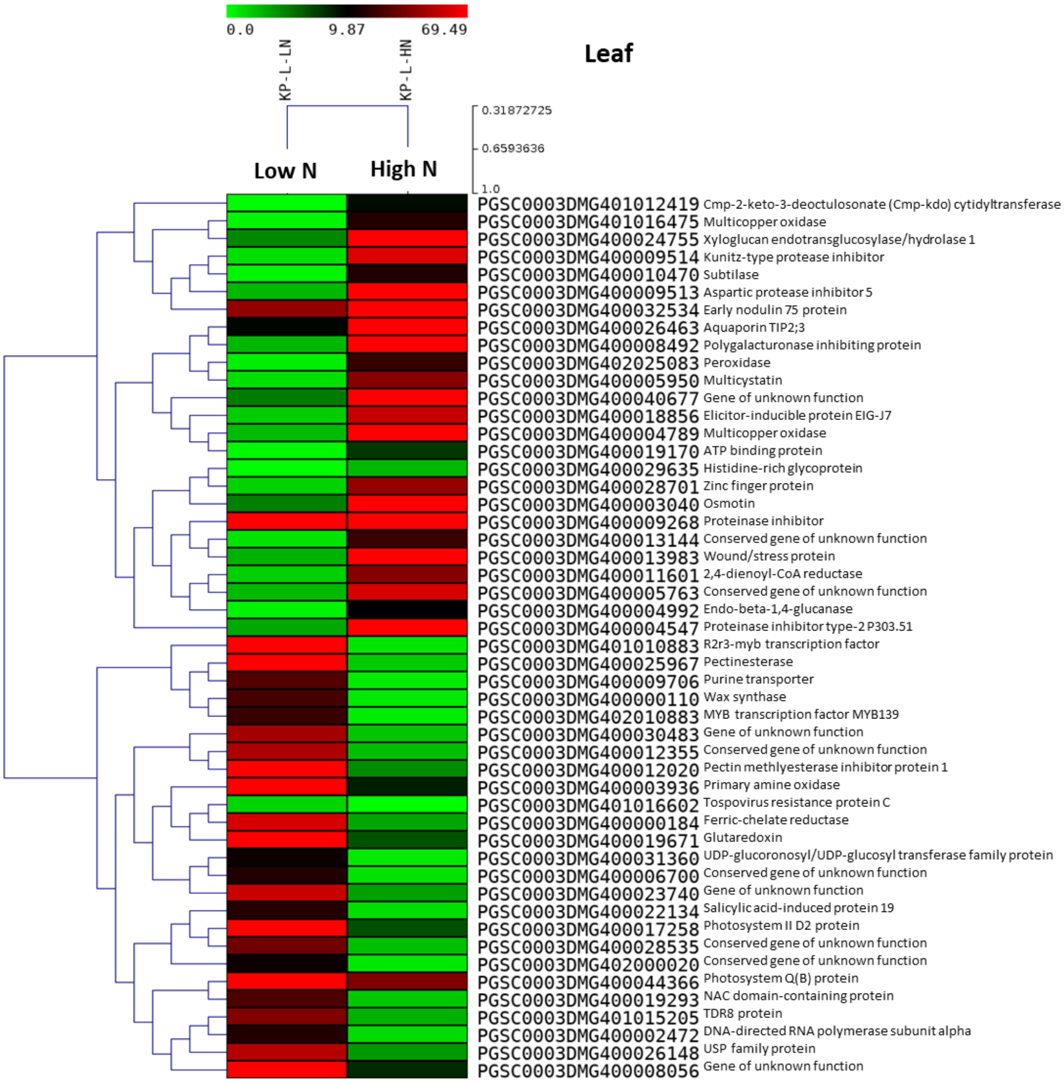

Fig. 6.6. Heat maps of the top 50 differentially expressed genes ($p < 0.05$) in leaf tissues of potato variety Kufri Pukhraj under high N (low N (control)) by RNA-seq. In a heat map, each horizontal line refers to a gene. Relatively up-regulated genes are shown in red colour, whereas down-regulated genes are shown in green colour.

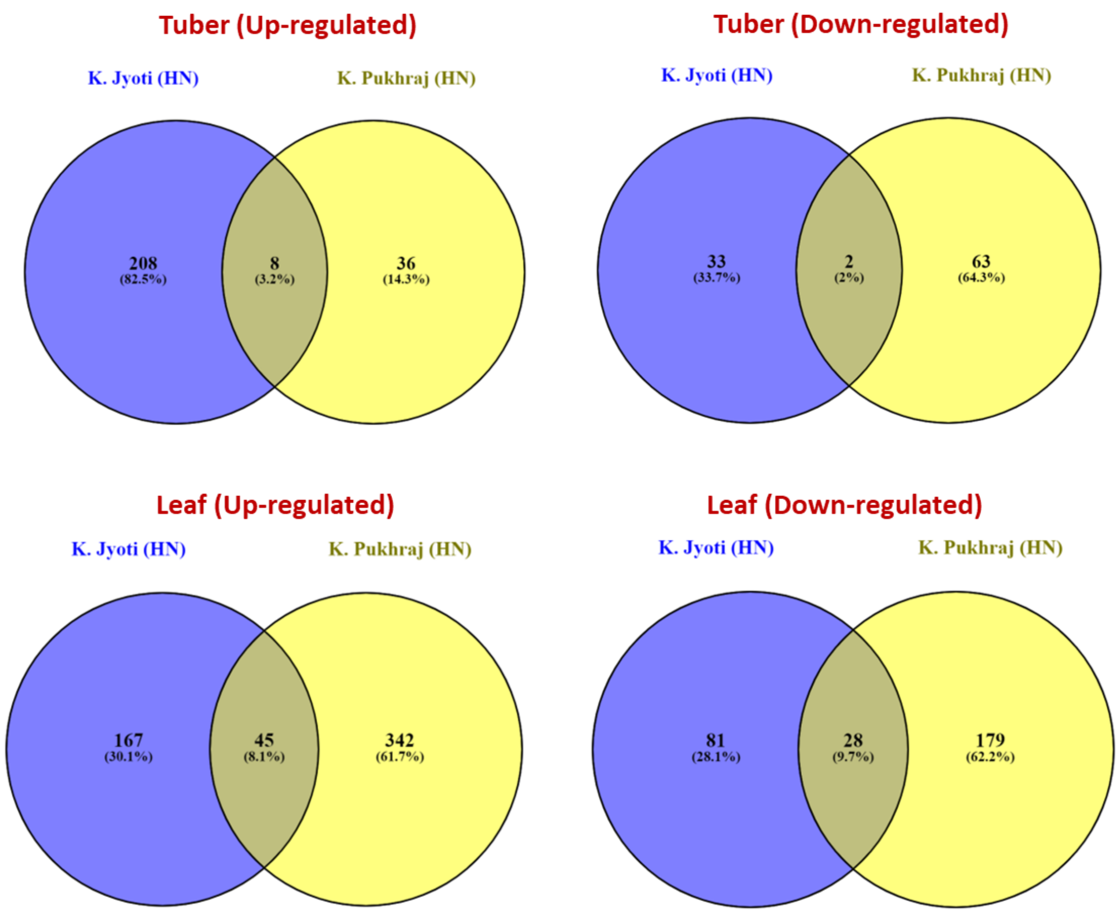

Fig. 6.7. Venn diagrams showing common genes (up-regulated and down-regulated) in potato varieties Kufri Jyoti and Kufri Pukhraj under high N versus (HN) low N (control) (LN) regimes.

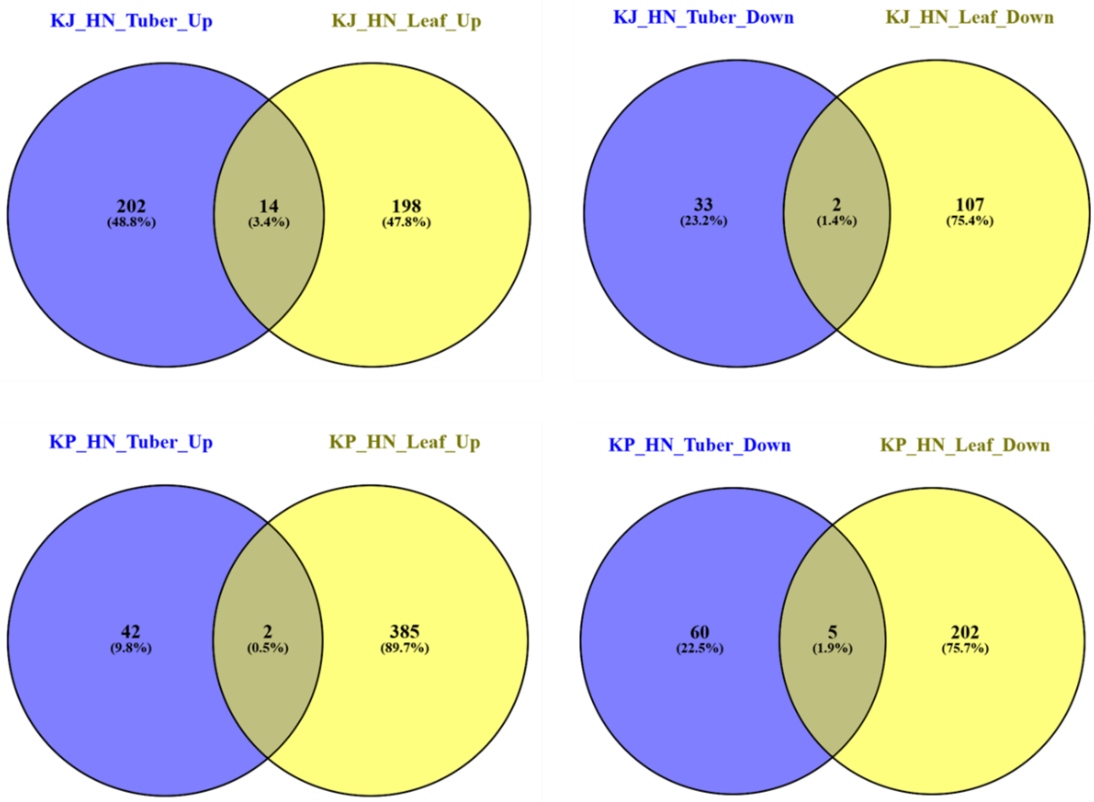

Fig. 6.8. Venn diagrams showing common genes (up-regulated and down-regulated) between tuber and leaf tissues of Kufri Jyoti and Kufri Pukhraj.

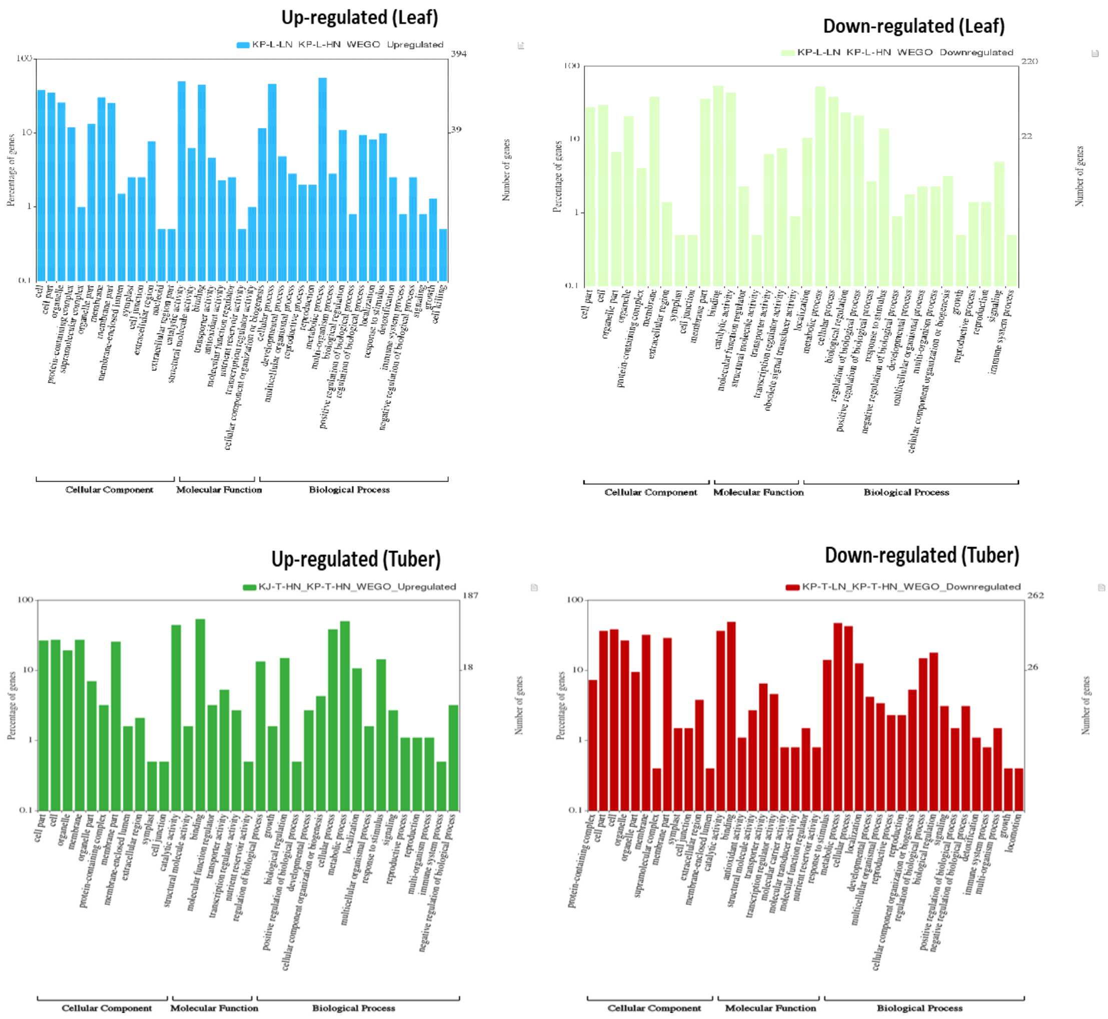

Fig. 6.9. Gene Ontology (GO) characterization for cellular component, molecular function, and biological process of up-regulated and down-regulated DEGs in potato cv. Kufri Pukhraj under high N versus low N (control).

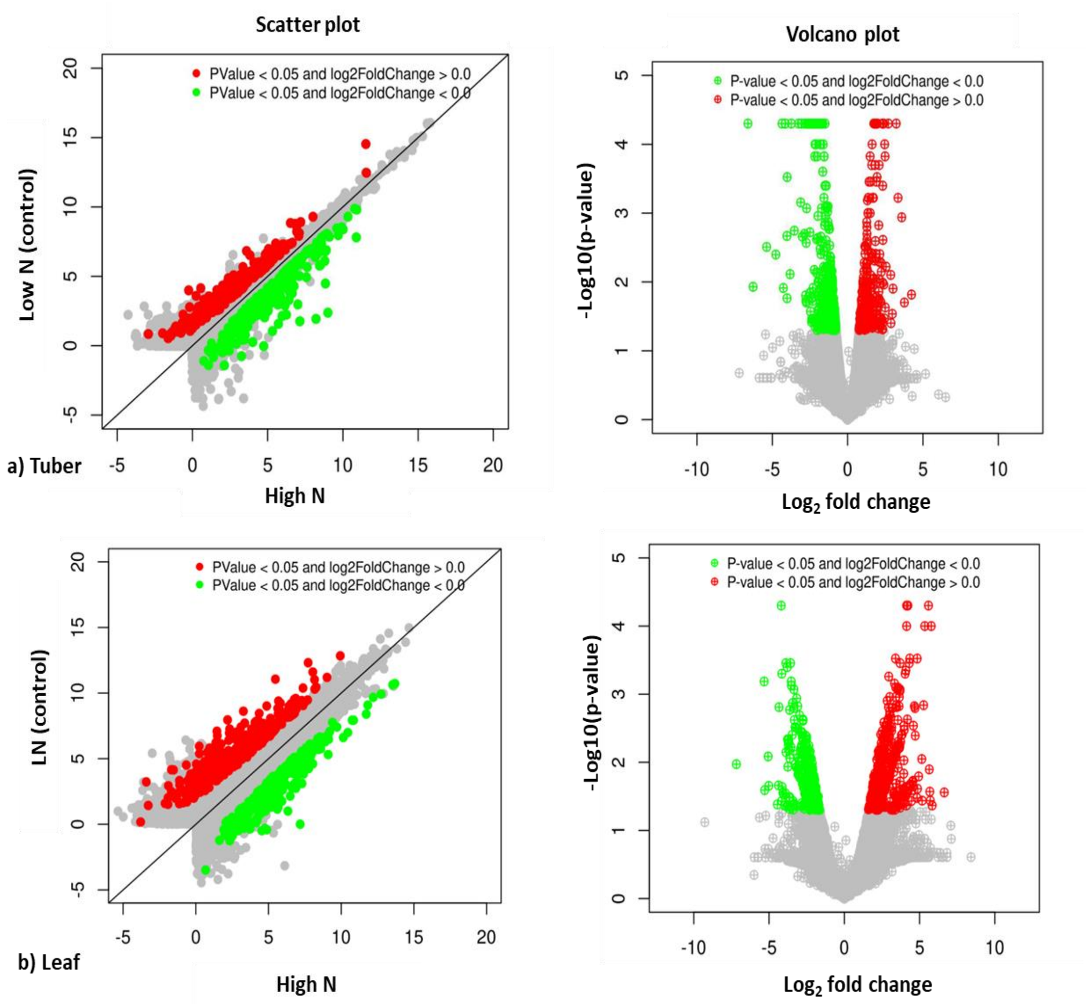

Fig. 6.10. Scatter plot and Volcano plot analysis of up-regulated and down-regulated DEGs in potato cv. Kufri Pukhraj under high N versus low N (control).

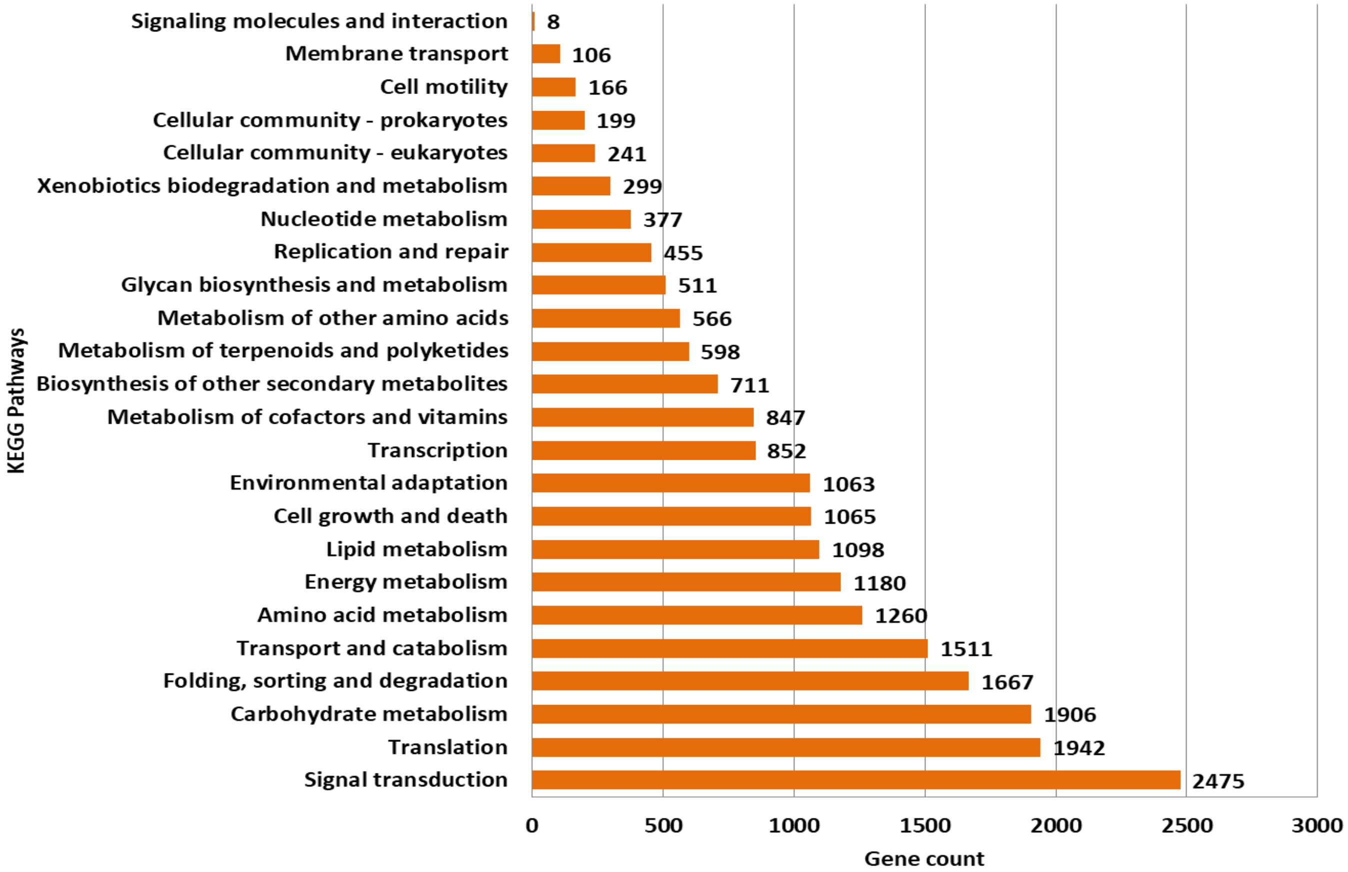

Fig. 6.11. KEGG pathways categorization of up-regulated and down-regulated DEGs in potato cv. Kufri Pukhraj under high N versus low N (control).

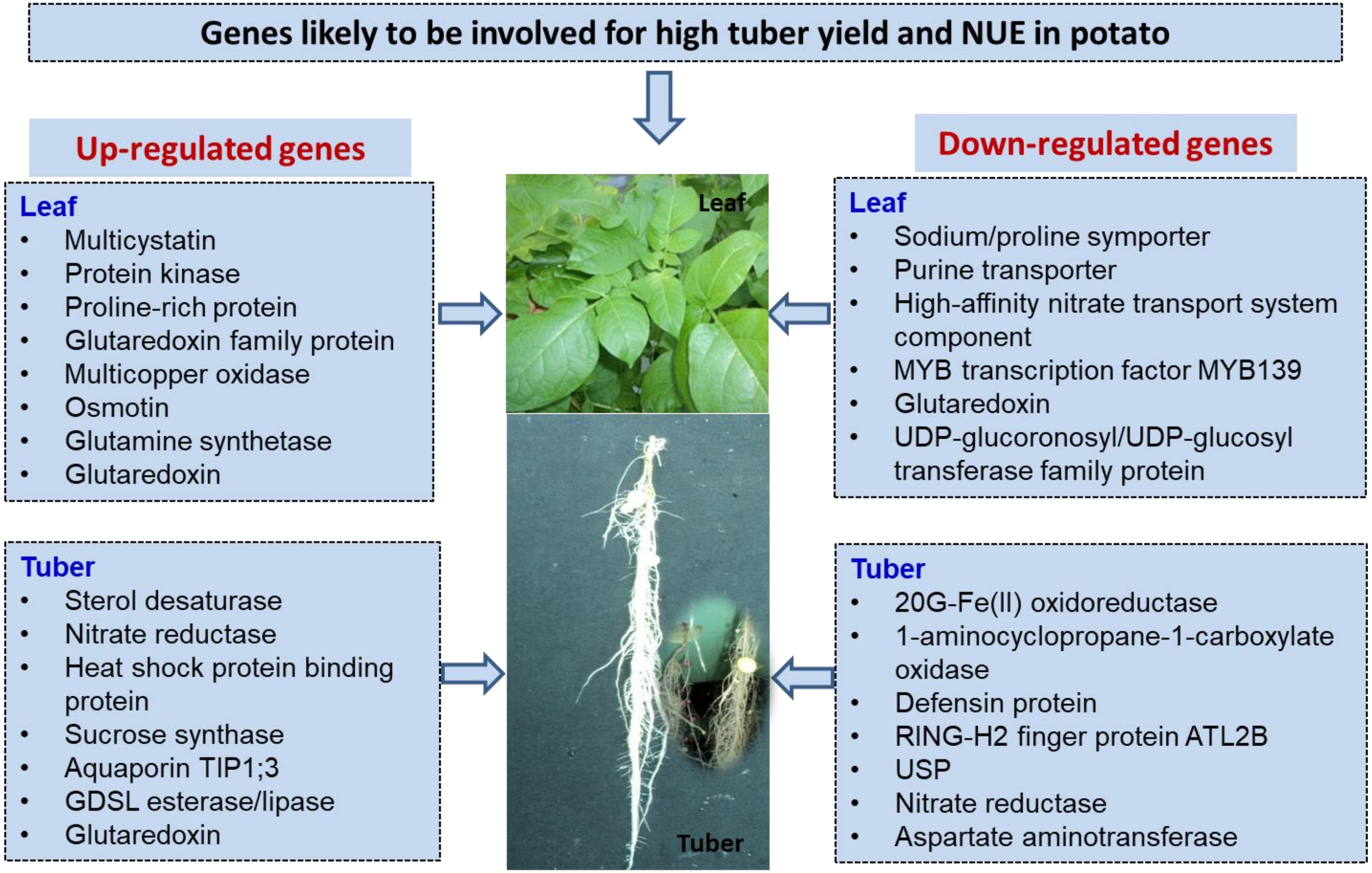

Fig. 6.12. A schematic view of differentially expressed genes likely to be involved in potato above-ground (leaf) and under-ground (tuber) plant parts for up-regulated and down-regulated genes in potato variety Kufri Pukhraj under high N for high tuber yield under aeroponics.

Table 6.15. Validation through qRT-PCR analysis

Sr. No	Gene ID	Gene regulation	Gene description	Gene ID	Primer sequence (5'→3')	Gene expression values (Log$_2$ FC)	
						RNA-seq	RT-qPCR
Tuber Tissue							
1.	Kufri Jyoti	Up-regulated	Nitrate reductase	PGSC0003DMG400030212	F: GTGTAGCTCTCATCCCAAGG R: TGCCAACAGGTAAGCCTAAG	4.25	3.87
2.		Down-regulated	20G-Fe(II) oxidoreductase	PGSC0003DMG400030362	F: CAAAGCACAAAGTACAACCCC R: AAGACCAGTTTTGAGGCCTAG	-3.64	-3.08
3.	Kufri Pukhraj	Up-regulated	Aquaporin TIP1;3	PGSC0003DMG400028182	F: GTATTTGCAGGTTCAGGTTCC R: CCTCCAGAAATGTTAGCCCC	4.24	4.02
4.		Down-regulated	RING-H2 finger protein ATL2B	PGSC0003DMG400027871	F: CTTTAGGAGGAGCGACAATAGG R: GGAGTAGCCCTGTTTCTGTTG	-4.76	-4.30
Leaf Tissue							
5.	Kufri Jyoti	Up-regulated	Multicystatin	PGSC0003DMG400005950	F: TTGGGTGAAAGAATGGGAGG R: AACAGCAAAACGAGCAAGATC	7.93	8.30
6.		Down-regulated	Sodium/proline symporter	PGSC0003DMG400009706	F: ACTAACCATTCACCAGCCTTC R: AGAATAAGTTGAGGCAGGAAGG	-5.60	-4.72
7.	Kufri Pukhraj	Up-regulated	Xyloglucan endotransglucosylase/ hydrolase 1	PGSC0003DMG400024755	F: CACTGCATTTTACCTGTCATCG R: TCTCTGTTCTCTGTTTCCTTTTCC	5.77	4.39
8.		Down-regulated	Purine transporter	PGSC0003DMG400009706	F: ACTAACCATTCACCAGCCTTC R: AGAATAAGTTGAGGCAGGAAGG	-5.29	-4.21

HN: High N, LN: Low N; RT-qPCR analysis was performed in HN versus LN (control) of the same variety.

6.3 Transcriptomics analysis uncovers genes underlying high tuber yields in potato varieties under aeroponics

6.3.1 Transcriptome sequencing and analysis

In another transcriptomics study, the Illumina platform generated transcriptome data from 16 samples. The high-quality data (QV > 25) for tuber tissues ranged from 4.36 to 5.79 Gb, while for leaf tissues, it ranged from 3.75 to 5.32 Gb across all four varieties (Table 6.16). Reference mapping with the available potato genome sequence revealed fair alignment results, with values ranging from 73.40% to 81.93% for tuber tissues and from 73.20% to 83.23% for leaf tissues across the varieties (Table 6.17). Statistically significant differentially expressed genes (DEGs) were analyzed in the tuber and leaf tissues of potato varieties (Kufri Frysona, Kufri Khyati, and Kufri Mohan) by comparing them to the control variety (Kufri Sutlej) (Table 6.17). In the tuber tissues of Kufri Frysona, a total of 18511 DEGs were identified, with 301 genes significantly up-regulated and 309 genes down-regulated. In the tubers of Kufri Khyati, out of a total of 18283 DEGs, 226 genes were significantly up-regulated and 326 genes were down-regulated. For Kufri Mohan, of a total of 18730 DEGs, 267 genes were up-regulated and 340 genes were down-regulated in the tuber tissues. A similar pattern was observed for significant DEGs in the leaf tissues of Kufri Frysona (394 up-regulated and 243 down-regulated), Kufri Khyati (337 up-regulated and 268 down-regulated), and Kufri Mohan (318 up-regulated and 297 down-regulated) (Table 6.17).

6.3.2 Identification of potential genes involved in yield-related traits in potato

The top 20 significant differentially expressed genes (DEGs) in the tuber and leaf tissues of different potato varieties are shown in Table 6.18 and 6.19, respectively, with 10 up-regulated and 10 down-regulated DEGs. The top 50 DEGs in the tuber (Fig. 6.16) and leaf (Fig. 6.17) tissues of Kufri Mohan, the best-performing variety in this aeroponics study, are shown in heat maps. Additional heat maps for other varieties can be found in figures: Figures 6.14 (tuber: Kufri Frysona), 6.15 (tuber: Kufri Khyati), 6.18 (leaf: Kufri Frysona), and 6.19 (leaf: Kufri Khyati). Venn diagram analysis revealed common DEGs among Kufri Frysona, Kufri Khyati, and Kufri Mohan. In tuber tissues, there were 57 up-regulated and 75 down-regulated genes (Fig. 6.18). In contrast, only a few DEGs were identified in leaf tissues, with 7 up-regulated and 6 down-regulated genes (Fig. 6.19).

In tuber tissues, several significant genes were found to be highly up-regulated (with a Log_2 fold change ranging from 3.3 to 8.4) across all three potato varieties studied. These consist of laccase, lipoxygenase, Kunitz-type tuber invertase inhibitor, cysteine protease inhibitor 1, 101 kDa heat shock protein, and chloroplastic catechol oxidase B (Tables 6.18 and 6.19). Furthermore, only two of the types have up-regulated levels of certain genes: transcription factor R2R3-MYB (in Kufri Frysona and Kufri Mohan), fatty acyl-CoA reductase 3 (in Kufri Frysona and Kufri Khyati), and chitin-binding lectin 1 (in Kufri Khyati and Kufri Mohan). Conversely, a few significant genes were found to be down-regulated (with a Log2 fold change ranging from -6.5 to -3.2), including glutathione S-transferase, zinc finger family protein, and phenylalanine ammonia-lyase 1 (in Kufri Frysona and Kufri Khyati), phosphoethanolamine N-methyltransferase (in Kufri Frysona and Kufri Mohan), auxin-induced protein X10A (in Kufri Khyati and Kufri Mohan), and GDSL esterase/lipase (in Kufri Khyati and Kufri Frysona). In leaf tissues, commonly up-regulated genes (with a Log_2 fold change of 2.9 to 7.8) include 2,4-dienoyl-CoA reductase and glucosyltransferase (in Kufri Frysona and Kufri Khyati). In Kufri Frysona and Kufri Mohan, ubiquitin-protein ligase was the only common down-regulated gene (with a Log_2 fold change of -4.2 to -3.6). Furthermore, numerous genes were either up-regulated or down-regulated in a single variety as well (Tables 6.18 and 6.19).

6.3.3 GO annotation, Scatter plot, Volcano plot and KEGG pathways characterization

As outlined in Table 6.20, differentially expressed genes (DEGs) were examined in three Gene Ontology (GO) domains: molecular function, biological process, and cellular component. The molecular function domain had the most genes in the tuber and leaf tissues of all three types, followed by the biological process and cellular component domains (Fig. 6.20). Cell part, cell, membrane, membrane part, catalytic activity, binding, metabolic process, cellular process, and response to stimuli were among the GO categories that were overrepresented. Significant genes are illustrated in scatter plots and volcano plots presented in Fig. 6.21. Additionally, DEGs were categorized into 24 functional groups based on KEGG pathways. Signal transduction, translation, folding, sorting and degradation, transport, catabolism, and carbohydrate metabolism were the most common KEGG pathways found (refer to Fig. 6.22, Table 6.21, and Table 6.22). Additionally, volcano plots are shown in Figures 6.20 and 6.21.

6.3.4 Validation of genes by qRT-PCR analysis

RT-qPCR analysis was used to validate twelve chosen genes, revealing gene expression values ranging from -6.5 to 7.4 Log_2 fold change. The transcriptome sequencing results, which varied from -5.2 to 5.4 Log_2 fold change, were in agreement with these values (Table 18). Laccase and phosphoethanolamine N-methyltransferase in Kufri Frysona, catechol oxidase B chloroplastic and zinc finger family protein in Kufri Khyati, and cysteine protease inhibitor 1 and gamma-aminobutyrate transaminase isoform 2 in Kufri Mohan were among the validated up-regulated and down-regulated genes found in tuber tissues. Likewise, in leaf tissues, the genes protein tyrosine phosphatase 1 (PTP-1) and auxin-induced protein 5NG4 were validated in Kufri Frysona; galactosyltransferase family protein and the 70 kDa subunit of replication protein A were validated in Kufri Khyati; and 2,4-dienoyl-CoA reductase along with BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 were validated in Kufri Mohan. There were some slight differences between the RT-qPCR and RNA-seq data in terms of gene expression fold change values. All things considered, the investigation verified that RNA sequencing has identified putative genes linked to tuber yield and its component traits in potatoes.

Table 6.16. RNA-seq data generation and reference mapping

Sr. No.	Combination	No. of Filtered Paired-end (PE) Reads	Total no. of bases	Data in Gb	Reference mapping (%)
Tuber					
1.	Kufri Frysona (R1)	1,46,03,020	4,36,11,84,907	4.36 Gb	73.50%
2.	Kufri Frysona (R2)	1,55,89,436	4,65,75,36,489	4.65 Gb	81.93%
3.	Kufri Khyati (R1)	1,45,94,080	4,36,06,18,535	4.36 Gb	73.40%
4.	Kufri Khyati (R2)	1,50,56,075	4,49,69,63,257	4.49 Gb	76.28%
5.	Kufri Mohan (R1)	1,93,91,679	5,79,04,92,013	5.79 Gb	78.10%
6.	Kufri Mohan (R2)	1,58,42,251	4,73,57,42,770	4.73 Gb	79.63%
7.	Kufri Satluj (R1)	1,53,50,770	4,58,34,06,149	4.58 Gb	76.20%
8.	Kufri Satluj (R2)	1,69,47,921	5,06,30,78,724	5.06 Gb	80.48%
Leaf					
9.	Kufri Frysona (R1)	1,58,71,581	4,74,24,55,412	4.74 Gb	82.70%
10.	Kufri Frysona (R2)	1,45,68,460	4,35,17,65,721	4.35 Gb	74.34%
11.	Kufri Khyati (R1)	1,77,99,290	5,32,00,68,013	5.32 Gb	77.90%
12.	Kufri Khyati (R2)	1,69,01,903	5,05,02,40,980	5.05 Gb	83.23%
13.	Kufri Mohan (R1)	1,25,55,149	3,75,21,69,072	3.75 Gb	73.20%
14.	Kufri Mohan (R2)	1,29,80,649	3,87,96,23,470	3.87 Gb	76.34%
15.	Kufri Satluj (R1)	1,53,01,028	4,57,21,57,394	4.57 Gb	74.20%
16.	Kufri Satluj (R2)	1,75,22,128	5,23,69,37,225	5.23 Gb	81.54%

Table 6.17. Differentially expressed genes (DEGs) summary

Combination	**Total DEGs**	**Significant DEGs ($p < 0.05$)**			
		Up-regulated	**Down-regulated**	**Exclusive (control)**	**Exclusive (Treatment)**
Tuber					
Kufri Frysona	18511	301	309	565	356
Kufri Khyati	18283	226	326	731	288
Kufri Mohan	18730	267	340	641	392
Leaf					
Kufri Frysona	17971	394	243	300	362
Kufri Khyati	17348	337	268	426	228
Kufri Mohan	17419	318	297	331	248

DEGs analysis was performed using a variety Kufri Satluj (control) i.e., Kufri Frysona vs. Kufri Sutlej, Kufri Khyati vs. Kufri Sutlej, Kufri Mohan vs. Kufri Sutlej, for both tissues (tuber and leaf) separately.

Table 6.18. Selected differentially expressed genes (DEGs) associated with tuber yield-related traits in tuber tissues of potato grown under aeroponics

Sr. No.	Gene name/ID	Chr.	Gene expression (Log2 FC)	Statistical *P* value	Gene description
i) Kufri Frysona					
Up-regulated					
1	PGSC0003DMG400010139	3	7.50	0.000	Cysteine protease inhibitor 1
2	PGSC0003DMG400029575	8	6.87	0.000	Catechol oxidase B, chloroplastic
3	PGSC0003DMG400030376	6	6.60	0.040	Laccase
4	PGSC0003DMG400007113	6	6.42	0.025	Fatty acyl-CoA reductase 3
5	PGSC0003DMG400020999	8	6.21	0.010	Lipoxygenase
6	PGSC0003DMG400023764	1	5.42	0.004	Globulin
7	PGSC0003DMG400010146	3	5.19	0.000	Kunitz-type tuber invertase inhibitor
8	PGSC0003DMG400024644	3	4.61	0.006	101 kDa heat shock protein
9	PGSC0003DMG400047074	8	4.61	0.000	BURP domain-containing protein

10	PGSC0003DMG400013965	10	4.48	0.046	Transcription factor R2R3-MYB
Down-regulated					
11	PGSC0003DMG400020028	7	-6.33	0.041	Specific tissue protein 2
12	PGSC0003DMG400014459	12	-5.72	0.003	Phosphoethanolamine N-methyltransferase
13	PGSC0003DMG401026939	6	-5.07	0.050	HVA22 e
14	PGSC0003DMG400002167	9	-4.75	0.034	Glutathion S-transferase
15	PGSC0003DMG400026860	4	-4.24	0.010	Ubiquitin-protein ligase
16	PGSC0003DMG400031457	3	-4.18	0.008	Phenylalanine ammonia-lyase 1
17	PGSC0003DMG400029165	6	-3.56	0.026	Zinc finger family protein
18	PGSC0003DMG400015767	10	-3.47	0.015	Myb-like transcription factor
19	PGSC0003DMG400007815	12	-3.37	0.023	GDSL esterase/lipase
20	PGSC0003DMG401004894	6	-3.27	0.019	Transcription factor R18
ii) Kufri Khyati					
Up-regulated					
21	PGSC0003DMG400029575	8	7.46	0.000	Catechol oxidase B, chloroplastic

22	PGSC0003DMG400010146	3	7.43	0.022	Kunitz-type tuber invertase inhibitor
23	PGSC0003DMG400007113	6	6.62	0.021	Fatty acyl-CoA reductase 3
24	PGSC0003DMG400030376	6	6.44	0.038	Laccase
25	PGSC0003DMG400020999	8	6.26	0.006	Lipoxygenase
26	PGSC0003DMG400010139	3	5.18	0.000	Cysteine protease inhibitor 1
27	PGSC0003DMG400019517	3	4.97	0.004	Chitin-binding lectin 1
28	PGSC0003DMG400009513	3	4.45	0.001	Aspartic protease inhibitor 5
29	PGSC0003DMG400024644	3	3.76	0.009	101 kDa heat shock protein
30	PGSC0003DMG400015054	5	3.30	0.013	Dehydration-responsive protein RD22
Down-regulated					
31	PGSC0003DMG400029165	6	-6.59	0.032	Zinc finger family protein
32	PGSC0003DMG400013981	3	-5.46	0.036	Proline transporter 2
33	PGSC0003DMG400007815	12	-5.22	0.050	GDSL esterase/lipase
34	PGSC0003DMG400026010	1	-5.13	0.048	Auxin-induced protein X10A
35	PGSC0003DMG400015536	11	-4.90	0.012	MYB1-2

36	PGSC0003DMG400002167	9	-4.68	0.036	Glutathion S-transferase
37	PGSC0003DMG400031457	3	-4.24	0.004	Phenylalanine ammonia-lyase 1
38	PGSC0003DMG400008524	7	-4.18	0.007	F-box family protein
39	PGSC0003DMG400001967	8	-4.14	0.001	White-brown-complex ABC transporter family
40	PGSC0003DMG400029207	9	-3.22	0.014	WRKY transcription factor 6
iii) Kufri Mohan					
Up-regulated					
41	PGSC0003DMG400020999	8	8.43	0.000	Lipoxygenase
42	PGSC0003DMG400010139	3	6.86	0.000	Cysteine protease inhibitor 1
43	PGSC0003DMG400030376	6	6.21	0.030	Laccase
44	PGSC0003DMG400029575	8	6.20	0.000	Catechol oxidase B, chloroplastic
45	PGSC0003DMG400019517	3	6.13	0.002	Chitin-binding lectin 1
46	PGSC0003DMG402031759	2	5.88	0.043	Phospholipase A1
47	PGSC0003DMG400010146	3	5.37	0.000	Kunitz-type tuber invertase inhibitor
48	PGSC0003DMG400024644	3	5.01	0.002	101 kDa heat shock protein

49	PGSC0003DMG400013965	10	4.88	0.028	Transcription factor R2R3-MYB
50	PGSC0003DMG400013966	10	4.82	0.039	MYB transcription factor
Down-regulated					
51	PGSC0003DMG400025228	8	-6.37	0.045	Gamma aminobutyrate transaminase isoform2
52	PGSC0003DMG403019771	6	-5.77	0.046	Cytochrome P450
53	PGSC0003DMG400028426	2	-5.60	0.039	Cellulose synthase catalytic subunit
54	PGSC0003DMG402024222	8	-5.41	0.001	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1
55	PGSC0003DMG400014459	12	-5.36	0.002	Phosphoethanolamine N-methyltransferase
56	PGSC0003DMG400013547	7	-5.19	0.000	Sucrose synthase
57	PGSC0003DMG400015482	2	-4.67	0.039	Transcription factor bHLH63
58	PGSC0003DMG400004438	10	-4.12	0.043	Aquaporin, MIP family, PIP subfamily
59	PGSC0003DMG400026010	1	-3.92	0.010	Auxin-induced protein X10A
60	PGSC0003DMG400000711	1	-3.75	0.037	Basic helix-loop-helix protein BHLH7

DEGs were analysed in comparison with potato variety Kufri Sutlej (control). FC: Fold change

Table 6.19. Selected differentially expressed genes (DEGs) associated with tuber yield-related traits in leaf tissues of potato grown under aeroponics

Sr. No.	Gene name/ID	Chr.	Gene expression (Log_2 FC)	Statistical *P* value	Gene description
i) Kufri Frysona					
Up-regulated					
1	PGSC0003DMG400030842	4	7.11	0.017	PTP-1
2	PGSC0003DMG400007335	2	6.21	0.000	Apyrase 3
3	PGSC0003DMG400011601	12	5.11	0.016	2,4-dienoyl-CoA reductase
4	PGSC0003DMG400030265	3	5.00	0.022	Glucosyltransferase
5	PGSC0003DMG400008309	2	4.73	0.000	Chlorophyll a/b binding protein
6	PGSC0003DMG400025194	1	4.55	0.022	Dehydration-responsive protein RD22
7	PGSC0003DMG400024863	4	4.10	0.025	Cytochrome P450 hydroxylase
8	PGSC0003DMG400028182	10	3.68	0.005	Aquaporin TIP1;3
9	PGSC0003DMG400021689	3	3.43	0.038	UDP-glucosyltransferase family 1 protein

10	PGSC0003DMG400028701	4	3.20	0.001	Zinc finger protein
Down-regulated					
11	PGSC0003DMG400031871	1	-6.29	0.018	Stress-associated protein 3
12	PGSC0003DMG400001958	8	-5.01	0.020	Auxin-induced protein 5NG4
13	PGSC0003DMG402010367	2	-4.53	0.025	Replication factor A protein
14	PGSC0003DMG400002213	4	-4.24	0.033	Aldehyde dehydrogenase
15	PGSC0003DMG403029631	9	-3.96	0.012	F-box family protein
16	PGSC0003DMG400004715	12	-3.91	0.022	Trichohyalin
17	PGSC0003DMG400026860	4	-3.90	0.010	Ubiquitin-protein ligase
18	PGSC0003DMG400019110	5	-3.50	0.002	Chalcone synthase 2
19	PGSC0003DMG400015151	3	-3.47	0.039	Cytochrome P450
20	PGSC0003DMG400019758	3	-3.32	0.008	Dihydrodipicolinate synthase, chloroplastic
ii) Kufri Khyati					
Up-regulated					
21	PGSC0003DMG401026939	6	7.81	0.021	HVA22 e

22	PGSC0003DMG400007301	6	4.92	0.028	TBZ17
23	PGSC0003DMG401012430	7	4.81	0.036	Galactosyltransferase family protein
24	PGSC0003DMG400005005	12	4.76	0.007	C-terminal zinc-finger
25	PGSC0003DMG400011601	12	4.69	0.024	2,4-dienoyl-CoA reductase
26	PGSC0003DMG402032203	1	4.44	0.022	Extensin
27	PGSC0003DMG400027963	6	3.67	0.001	GA20 oxidase
28	PGSC0003DMG400020829	6	3.61	0.023	Copper transporter 1
29	PGSC0003DMG400008309	2	3.34	0.000	Chlorophyll a/b binding protein
30	PGSC0003DMG400029350	12	2.99	0.003	Glycosyltransferase
Down-regulated					
31	PGSC0003DMG400002426	6	-4.79	0.027	Resistance gene
32	PGSC0003DMG400020377	12	-4.60	0.000	70 kDa subunit of replication protein A
33	PGSC0003DMG400011752	7	-3.89	0.030	Cellulose synthase
34	PGSC0003DMG400008372	10	-3.85	0.026	VQ motif family protein
35	PGSC0003DMG400031091	7	-3.76	0.000	Glutathione S-transferase

36	PGSC0003DMG400024478	3	-3.68	0.035	Calmodulin-binding protein
37	PGSC0003DMG400026148	7	-3.55	0.001	USP family protein
38	PGSC0003DMG400017713	4	-3.42	0.042	LRR receptor-like serine/threonine-protein kinase
39	PGSC0003DMG400029623	9	-3.27	0.034	Salicylic acid/benzoic acid carboxyl methyltransferase
40	PGSC0003DMG400029207	9	-3.09	0.001	WRKY transcription factor 6
iii) Kufri Mohan					
Up-regulated					
41	PGSC0003DMG401026939	6	7.81	0.026	HVA22 e
42	PGSC0003DMG400015707	8	5.15	0.041	Gene of unknown function
43	PGSC0003DMG400011601	12	5.08	0.026	2,4-dienoyl-CoA reductase
44	PGSC0003DMG401015877	4	4.58	0.006	NBS-LRR protein
45	PGSC0003DMG400025587	4	4.58	0.030	ATP-citrate synthase
46	PGSC0003DMG400031848	9	4.41	0.004	CXE carboxylesterase
47	PGSC0003DMG400025967	1	4.04	0.001	Pectinesterase
48	PGSC0003DMG400031844	9	3.67	0.043	2-Hydroxyisoflavanone dehydratase

49	PGSC0003DMG400015437	12	3.34	0.047	UDP-glucose glucosyltransferase
50	PGSC0003DMG400030181	4	3.07	0.000	Esterase/lipase/thioesterase family protein
Down-regulated					
51	PGSC0003DMG402024222	8	-6.59	0.001	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1
52	PGSC0003DMG400012100	7	-6.18	0.000	Major latex
53	PGSC0003DMG400008517	5	-4.86	0.037	MtN3 protein
54	PGSC0003DMG400017189	9	-4.48	0.015	Desacetoxyvindoline 4-hydroxylase
55	PGSC0003DMG400021142	2	-3.97	0.023	DWARF1/DIMINUTO
56	PGSC0003DMG400024278	12	-3.70	0.008	Phenylacetaldehyde synthase
57	PGSC0003DMG401027561	4	-3.60	0.039	Ubiquitin-protein ligase
58	PGSC0003DMG400021458	2	-3.59	0.009	ALA-interacting subunit 5
59	PGSC0003DMG400014293	3	-3.51	0.011	Low-temperature-induced 65 kDa protein
60	PGSC0003DMG400000408	11	-3.13	0.003	Xyloglucan endotransglucosylase-hydrolase XTH6

DEGs were analysed in comparison with potato variety Kufri Sutlej (control). FC: Fold change

Table 6.20. GO annotation summary

Combination	Description	Biological Process	Cellular Component	Molecular Function
Tuber				
Kufri Frysona	Down-regulated	155	118	174
	Exclusive control	84	64	103
	Exclusive treated	186	143	223
	Expressed both	8870	7979	10782
	Up-regulated	192	126	215
Kufri Khyati	Down-regulated	167	146	197
	Exclusive control	63	61	82
	Exclusive treated	265	226	313
	Expressed both	8788	7867	10685
	Up-regulated	148	97	161
Kufri Mohan	Down-regulated	180	144	198
	Exclusive control	111	98	122
	Exclusive treated	210	182	246
	Expressed both	8985	8007	10925
	Up-regulated	167	115	197
Leaf				
Kufri Frysona	Down-regulated	114	90	143
	Exclusive control	111	66	124
	Exclusive treated	78	70	98
	Expressed both	8674	7757	10360
	Up-regulated	235	175	270
Kufri Khyati	Down-regulated	146	118	163

	Exclusive control	72	44	77
	Exclusive treated	107	102	138
	Expressed both	8435	7514	10074
	Up-regulated	176	147	197
Kufri Mohan	Down-regulated	165	113	193
	Exclusive control	78	53	76
	Exclusive treated	80	70	97
	Expressed both	8440	7575	10097
	Up-regulated	147	118	183

Table 6.21. KEGG Annotation Statistics

Combination	Identified gene count	KEGG Annotated gene count
Tuber		
Kufri Frysona	19656	5446
Kufri Khyati	19527	5419
Kufri Mohan	19993	5493
Leaf		
Kufri Frysona	18850	5266
Kufri Khyati	18217	5180
Kufri Mohan	18213	5175

Table 6.22. KEGG Pathway classification

KEGG pathways	Tuber			Leaf		
	Kufri Frysona	Kufri Khyati	Kufri Mohan	Kufri Frysona	Kufri Khyati	Kufri Mohan
Metabolism						
Carbohydrate metabolism	493	487	498	477	469	462
Energy metabolism	286	285	289	298	302	298
Lipid metabolism	285	284	290	279	279	276
Nucleotide metabolism	97	96	98	93	93	94
Amino acid metabolism	330	326	331	311	309	304
Metabolism of other amino acids	155	152	154	141	133	137
Glycan biosynthesis and metabolism	129	130	131	129	129	129
Metabolism of cofactors and vitamins	213	214	214	217	215	216
Metabolism of terpenoids and polyketides	161	159	162	154	151	154
Biosynthesis of other secondary metabolites	198	199	201	160	151	157
Xenobiotics biodegradation and metabolism	89	87	90	69	67	69

KEGG pathways	Tuber			Leaf		
	Kufri Frysona	Kufri Khyati	Kufri Mohan	Kufri Frysona	Kufri Khyati	Kufri Mohan
Genetic Information Processing						
Transcription	219	214	218	211	209	209
Translation	488	492	494	479	486	481
Folding, sorting and degradation	427	421	425	414	410	411
Replication and repair	121	121	121	111	105	102
Environmental Information Processing						
Membrane transport	28	28	28	24	24	22
Signal transduction	636	631	639	626	606	609
Signaling molecules and interaction	2	2	2	2	2	2
Cellular Processes						
Transport and catabolism	387	388	391	380	375	378
Cell growth and death	278	277	279	270	254	257
Cellular community – eukaryotes	62	63	63	63	61	61
Cellular community – prokaryotes	52	51	52	48	47	45
Cell motility	42	42	44	42	41	42
Organismal Systems						
Environmental adaptation	268	270	279	268	262	260

Table 6.23. Validation of selected genes through qRT-PCR analysis

Gene ID	Tissue	Gene ID	Gene description	Primer sequence (5'→3')	qPCR amplicon length (bp)	Gene expression (Log$_2$ FC)	
						RNA-seq	RT-qPCR
Tuber							
Kufri Frysona	Tuber	PGSC0003DMG400030376	Laccase	F: TGTCATTATGGTTGGACCTGG R: TTGTACTCAAGGATGGCAGTG	150	6.60	4.87
	Tuber	PGSC0003DMG400014459	Phosphoethanolamine N-methyltransferase	F: TGGAGCATACATCGGAACTTAC R: AGCACCCAACTCTAACACTG	137	-5.72	-5.28
Kufri Khyati	Tuber	PGSC0003DMG400029575	Catechol oxidase B, chloroplastic	F: TTACCGTGTGAAAGTCCGTG R: CGCTGTATTCACTTTTCCTGC	127	7.46	5.47
	Tuber	PGSC0003DMG400007815	GDSL esterase/lipase	F: ACGAAAGGATTTGGGCCTAG R: AACTTGATCTTGACCCTGAGC	143	-6.59	-4.72
Kufri Mohan	Tuber	PGSC0003DMG400010139	Cysteine protease inhibitor 1	F: AAACCTTCAATGCCCAAACG R: ACCACATCACCATAATCCGAC	114	6.86	4.83
	Tuber	PGSC0003DMG400025228	Gamma aminobutyrate transaminase isoform2	F: GCGGATGAGGTGATATGTGG R: TGCCAAAGAGACAAGATCAGG	121	-6.37	-5.20
Leaf							

Kufri Frysona	Leaf	PGSC0003DMG400030842	Protein tyrosine phosphatase (PTP)-1	F: GGCTGTGGGATTAGGTGTAAC R: ACATCGTCCGCCACTTTT	129	7.11	5.27
	Leaf	PGSC0003DMG400001958	Auxin-induced protein 5NG4	F: CTTGCCCCAATCGCTTATTTC R: AAGAATGACAGTACGAGCCG	134	-5.01	-3.87
Kufri Khyati	Leaf	PGSC0003DMG401012430	Galactosyltransferase family protein	F: CCCTCACTCCCAAACATACC R: GAGAAAATACTCCTTCCCGAGC	112	4.81	3.84
	Leaf	PGSC0003DMG400020377	70 kDa subunit of replication protein A	F: TCTCACTAACTTCATACGGCAAG R: GAATGCGGTCAAAGGTTGTG	150	-4.60	-4.20
Kufri Mohan	Leaf	PGSC0003DMG400011601	2,4-dienoyl-CoA reductase	F: CAGAGAAGGAGTGGGACAATG R: ATACTCCCCGATTCAAACCAG	150	5.08	4.62
	Leaf	PGSC0003DMG402024222	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1	F: TCTGGCATATTTACACGAGGC R: TGTAATGTGACTCTTCCCTGC	148	-6.59	-4.52

RT-qPCR was analysed in the potato varieties (Kufri Frysona, Kufri Khyati and Kufri Mohan) versus Kufri Satluj (control).

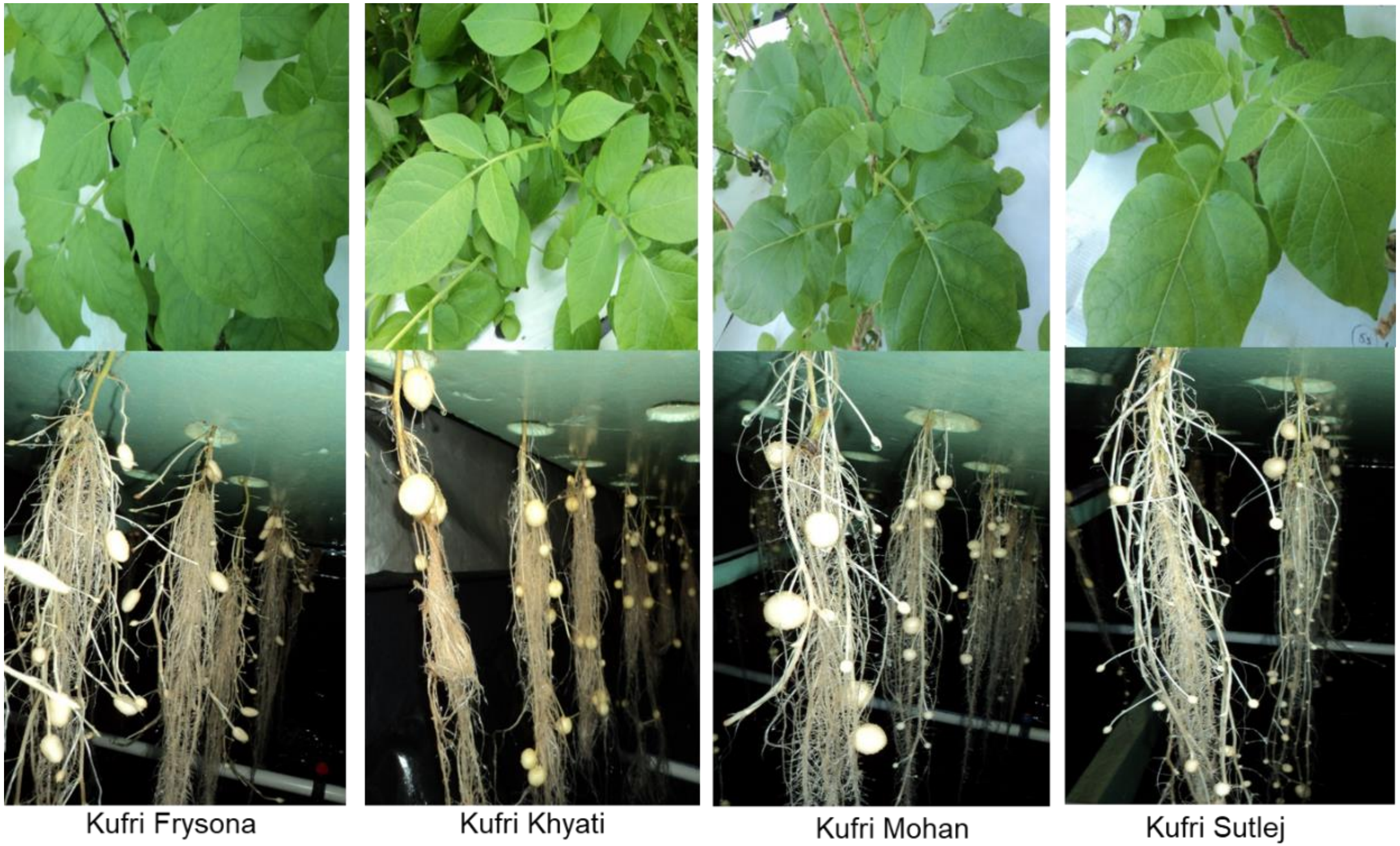

Fig. 6.13. Phenotypic traits and tuberization trend in 50-day-old potato plants of varieties, namely Kufri Frysona, Kufri Khyati, Kufri Mohan, and Kufri Sutlej (control), grown under aeroponic conditions in the Shimla hill area.

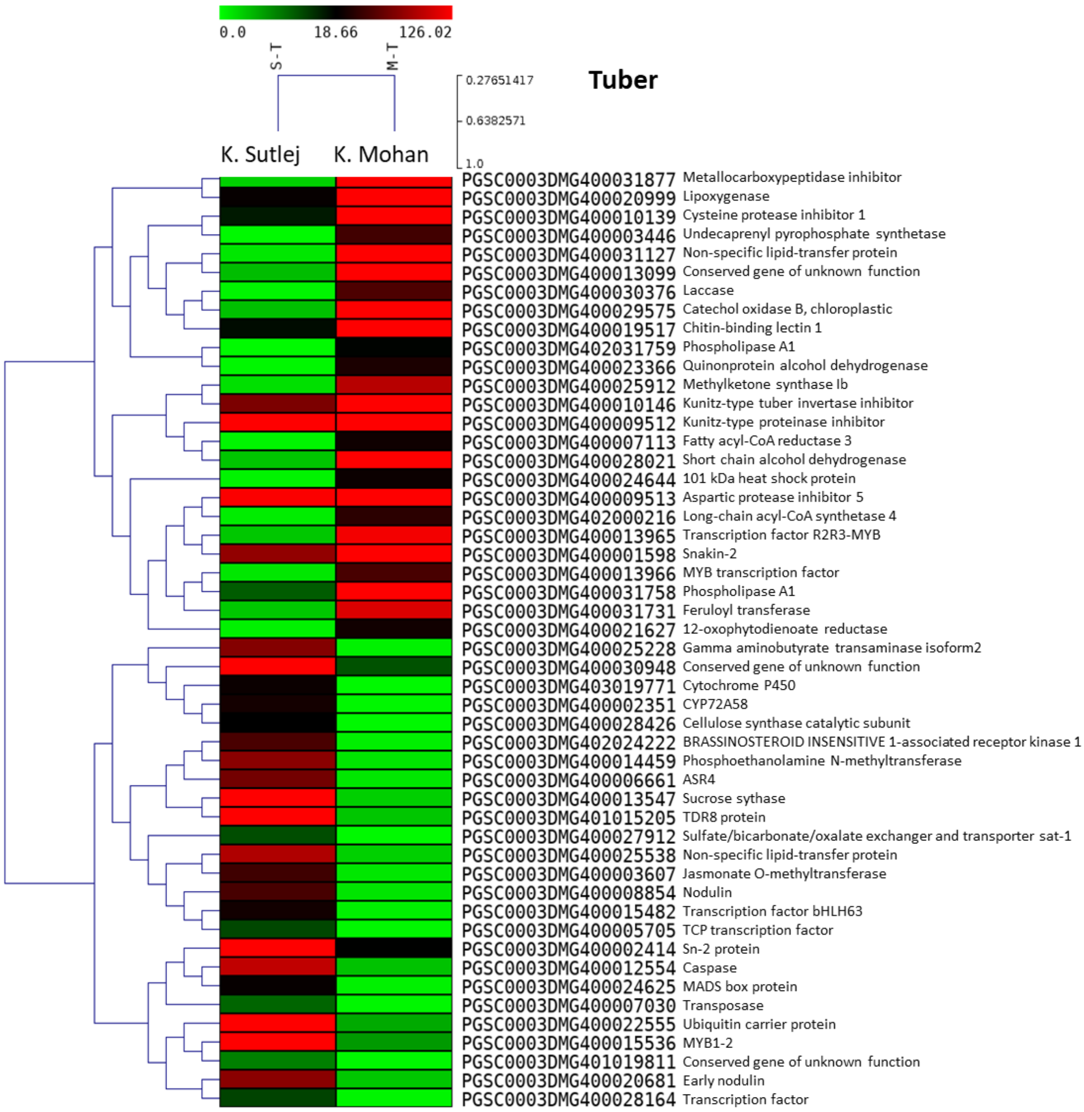

Fig 6.14. Heat maps of the top 50 differentially expressed genes (P < 0.05) in tuber tissues of potato variety Kufri Mohan versus Kufri Sutlej (control) associated with tuber yield-related traits under aeroponics. In a heat map, each horizontal line refers to a gene. Relatively up-regulated and down-regulated genes are shown in red and green colour, respectively.

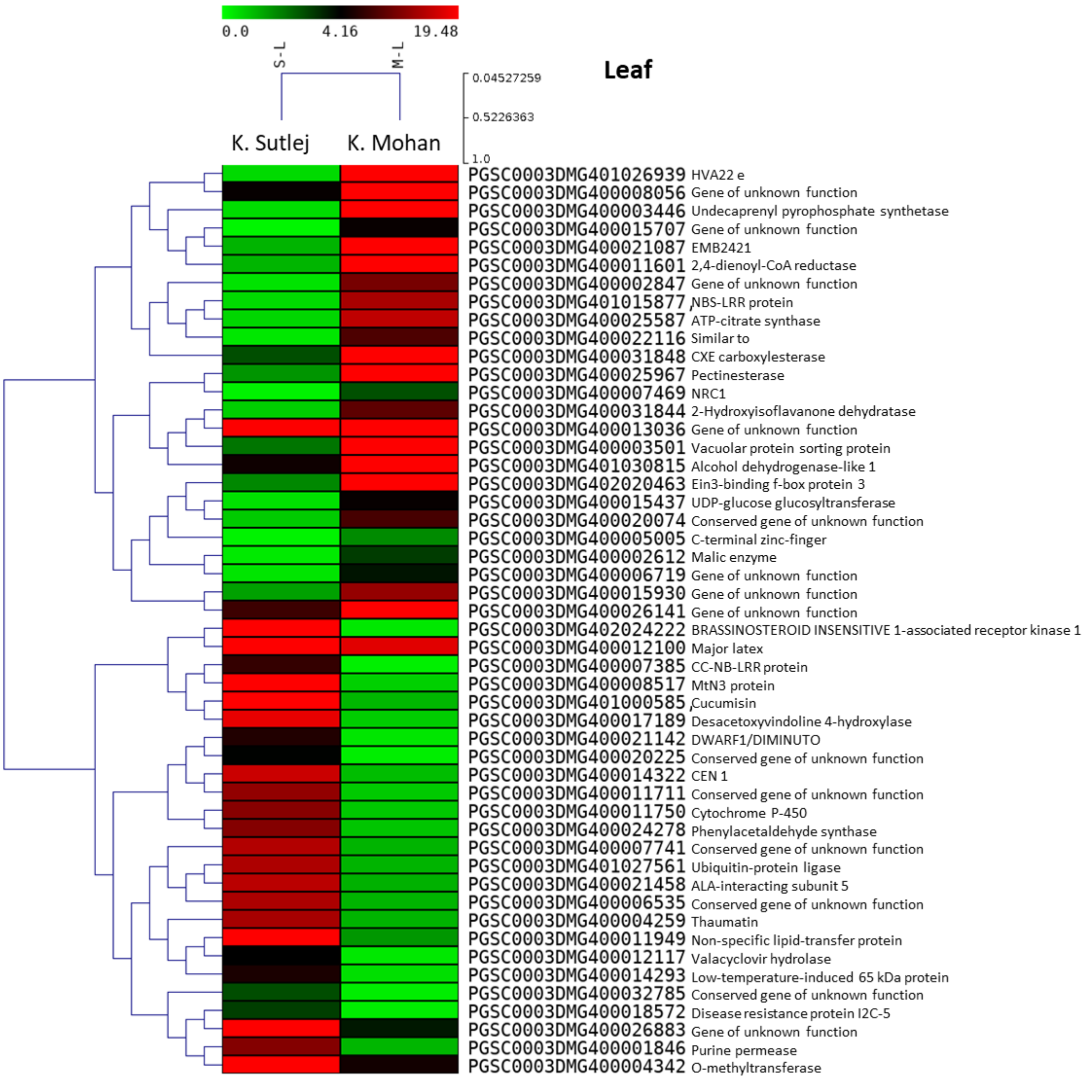

Fig.6.15. Heat maps of the top 50 differentially expressed genes ($P < 0.05$) in leaf tissues of potato variety Kufri Mohan versus Kufri Sutlej (control) associated with tuber yield-related traits under aeroponics. In a heat map, each horizontal line refers to a gene. Relatively up-regulated and down-regulated genes are shown in red and green colour, respectively.

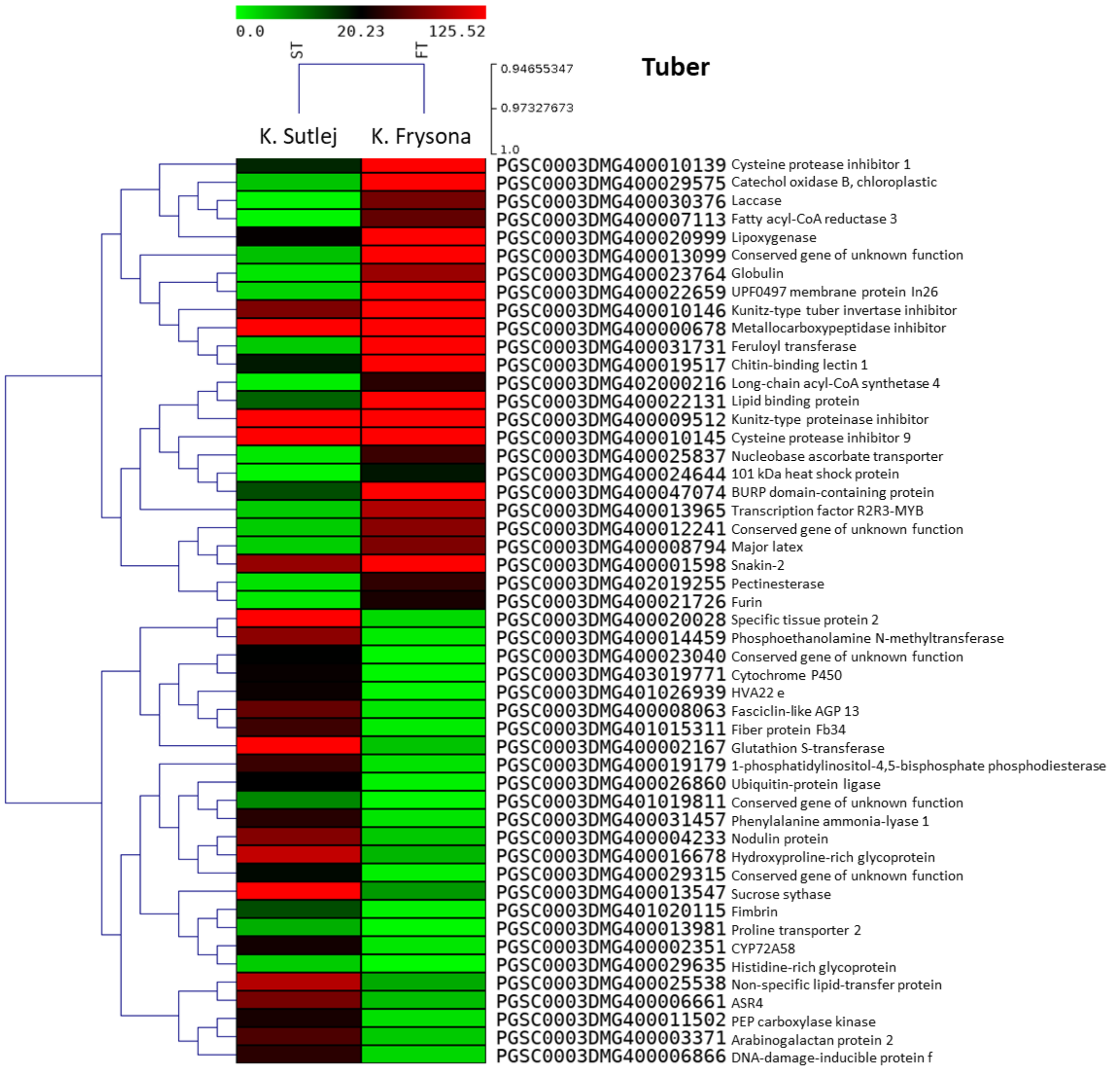

Fig.6.16. Heat maps of the top 50 differentially expressed genes ($P < 0.05$) in tuber tissues of potato varieties Kufri Frysona versus Kufri Sutlej (control) associated with tuber yield-related traits under aeroponics.

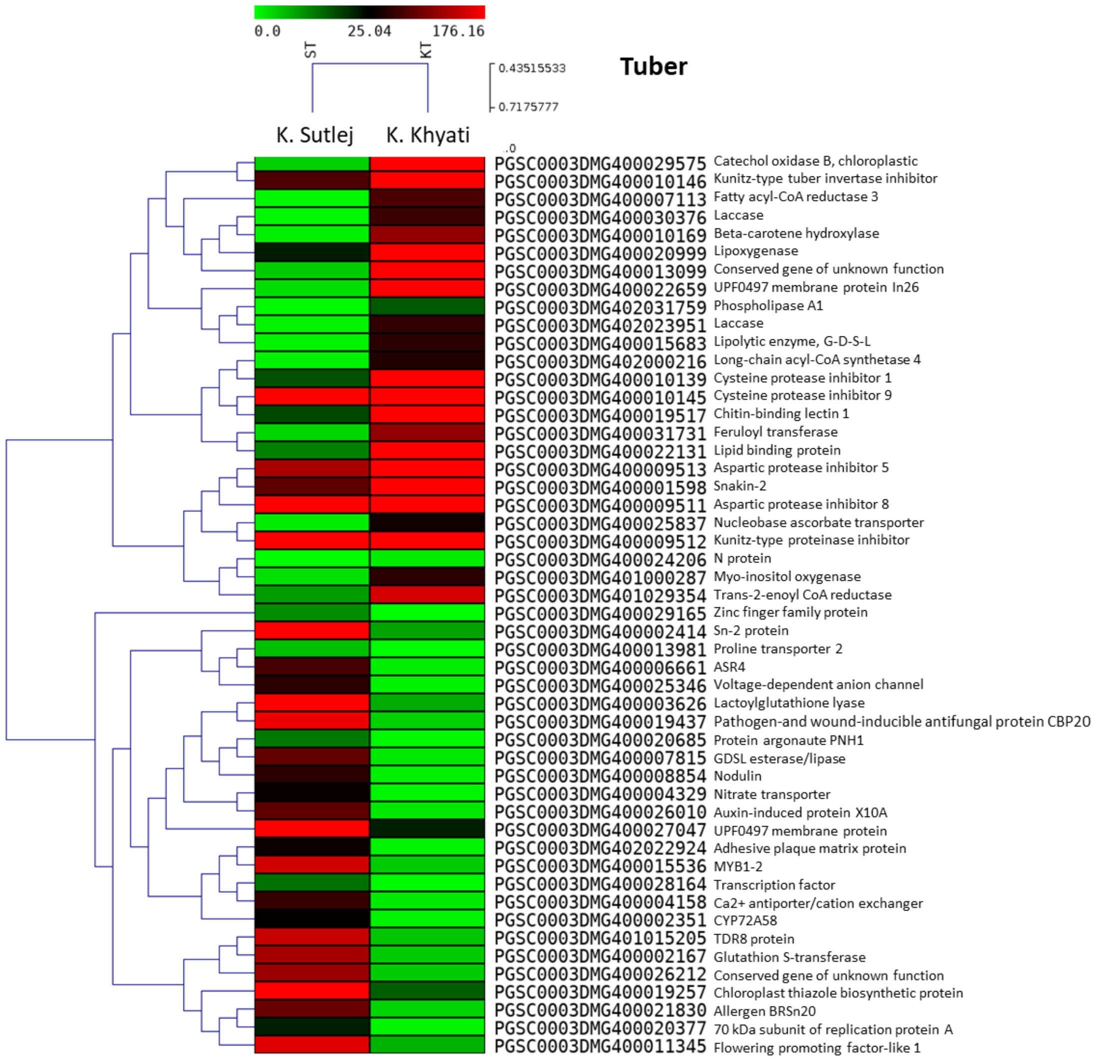

Fig. 6.17. Heat maps of the top 50 differentially expressed genes ($P < 0.05$) in tuber tissues of potato varieties Kufri Khyati versus Kufri Sutlej (control) associated with tuber yield-related traits under aeroponics.

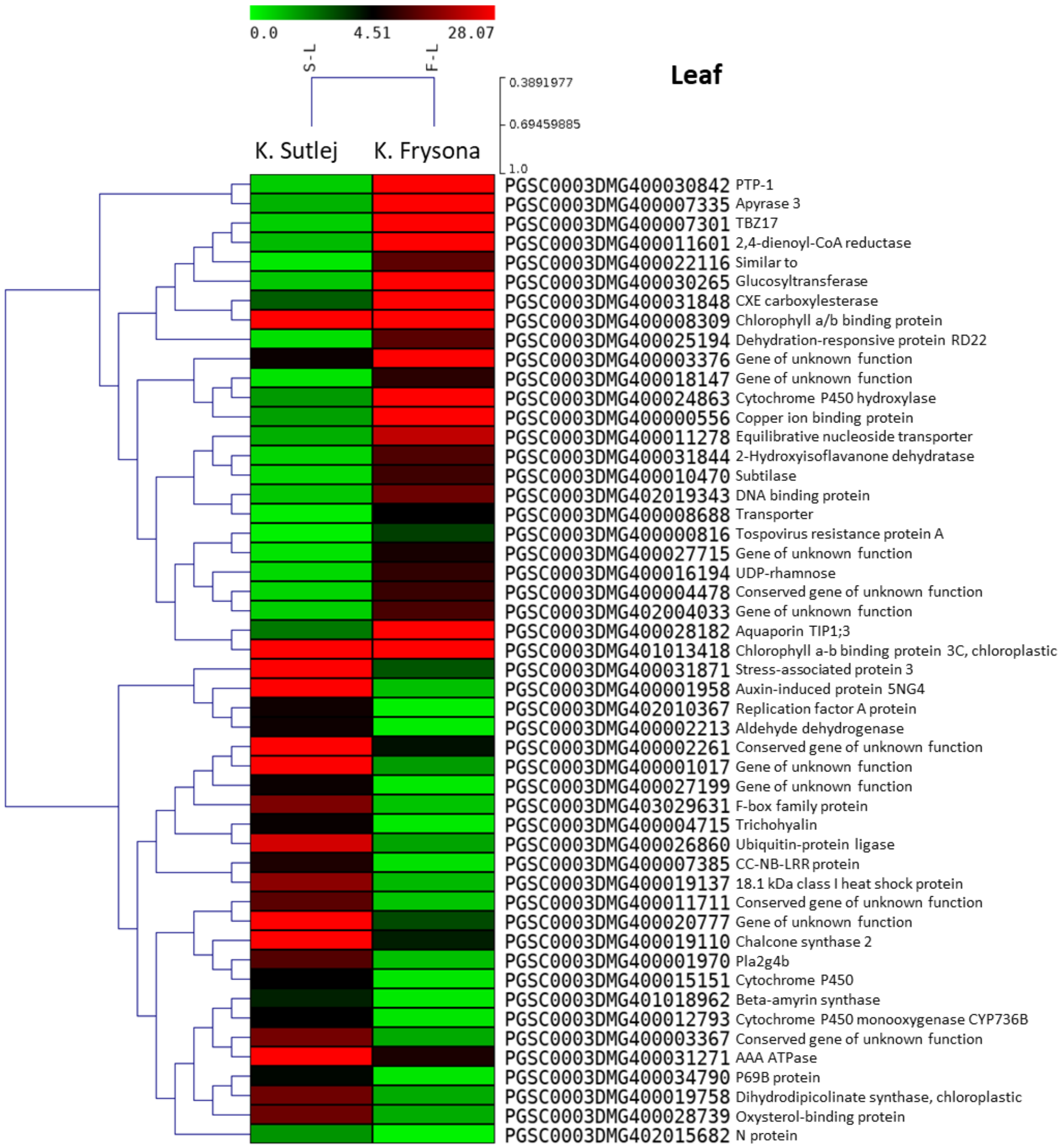

Fig. 6.18. Heat maps of the top 50 differentially expressed genes ($P < 0.05$) in leaf tissues of potato varieties Kufri Frysona versus Kufri Sutlej (control) associated with tuber yield-related traits under aeroponics.

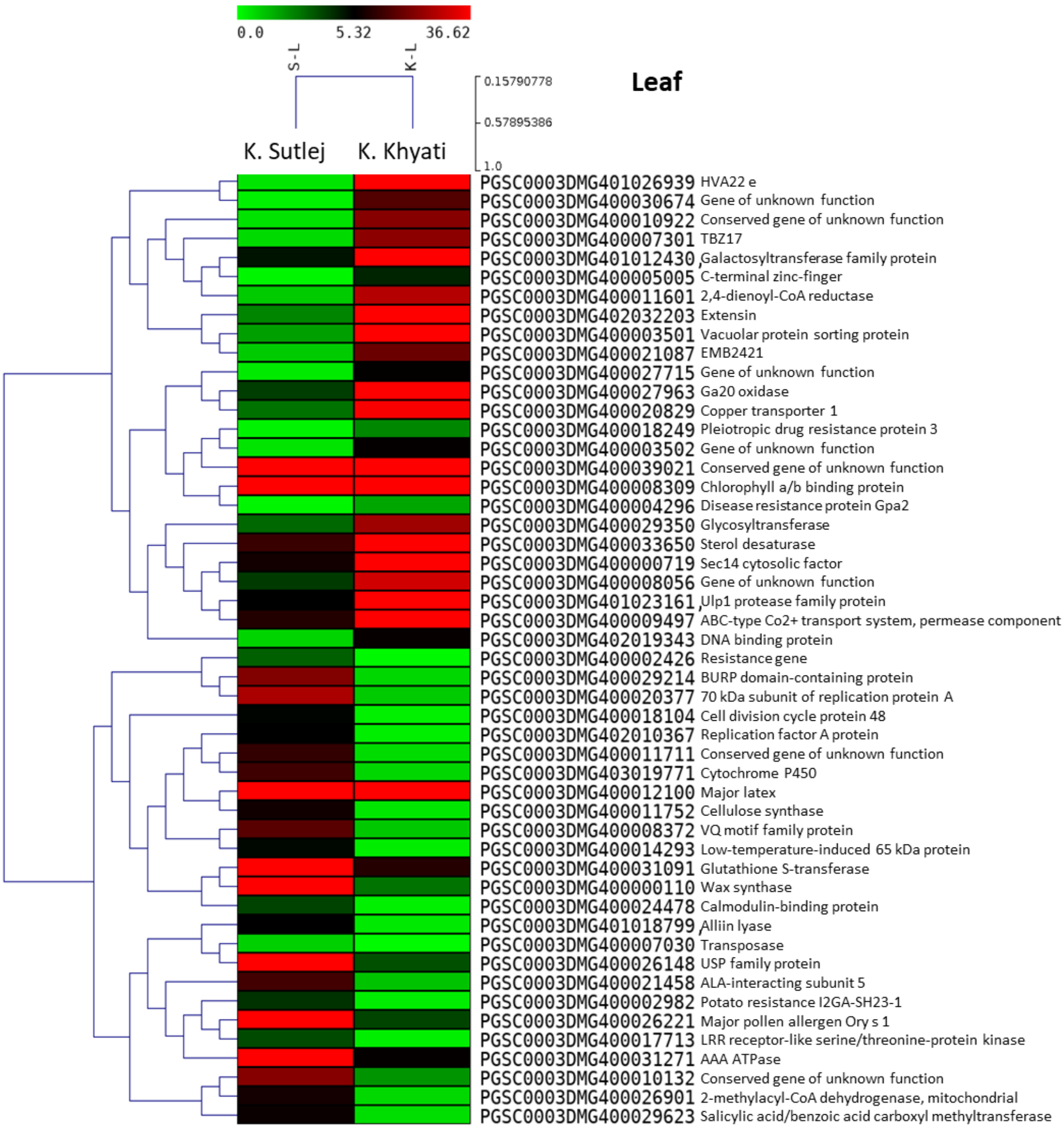

Fig. 6.19. Heat maps of the top 50 differentially expressed genes ($P < 0.05$) in leaf tissues of potato varieties Kufri Khyati versus Kufri Sutlej (control) associated with tuber yield-related traits under aeroponics.

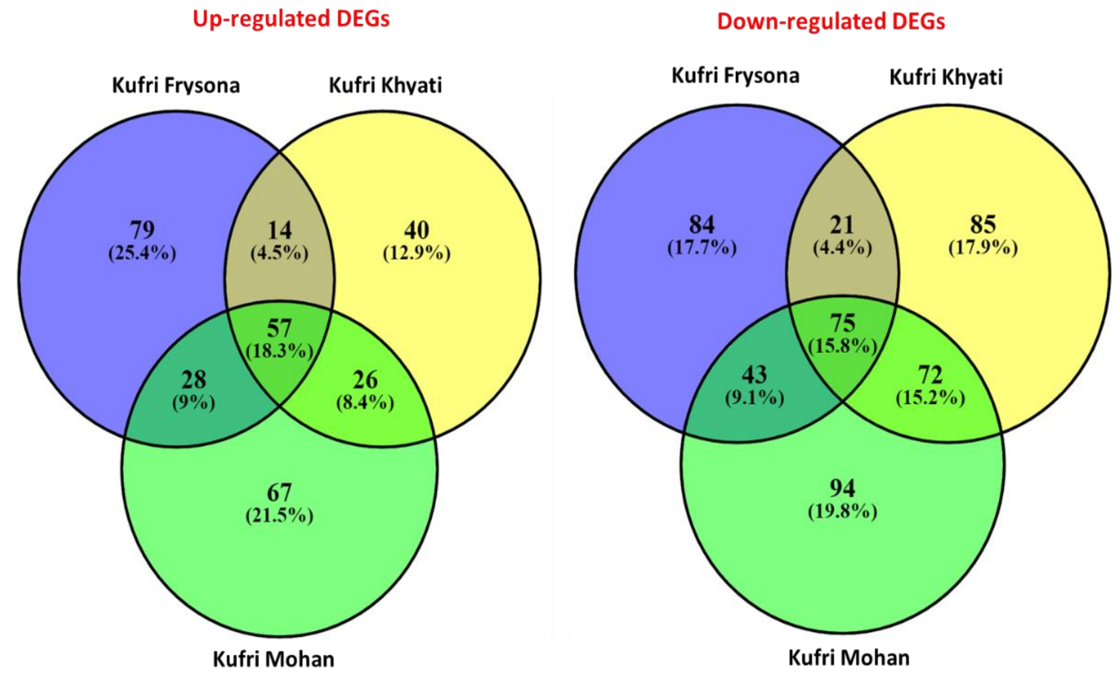

Fig. 6.20. Venn diagram showing common differentially expressed genes (DEGs) in the tuber tissues of potato varieties *viz.* Kufri Frysona, Kufri Khyati, Kufri Mohan, and Kufri Sutlej (control) under aeroponics.

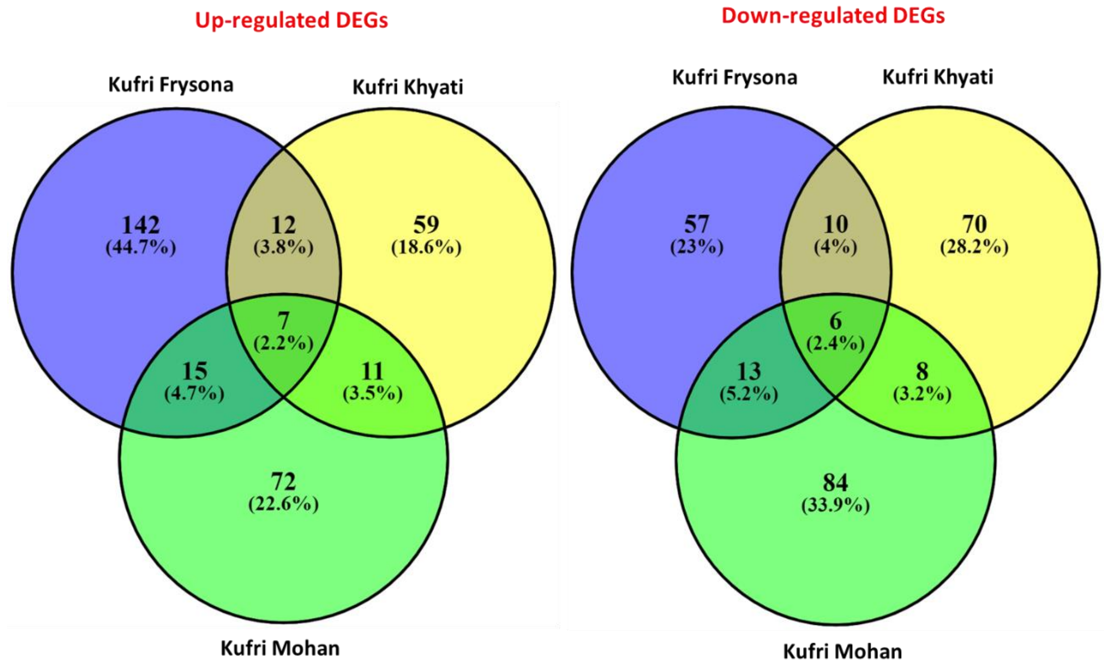

Fig. 6.21. Venn diagram showing common differentially expressed genes (DEGs) in the leaf tissues of potato varieties *viz.* Kufri Frysona, Kufri Khyati, Kufri Mohan, and Kufri Sutlej (control) under aeroponics.

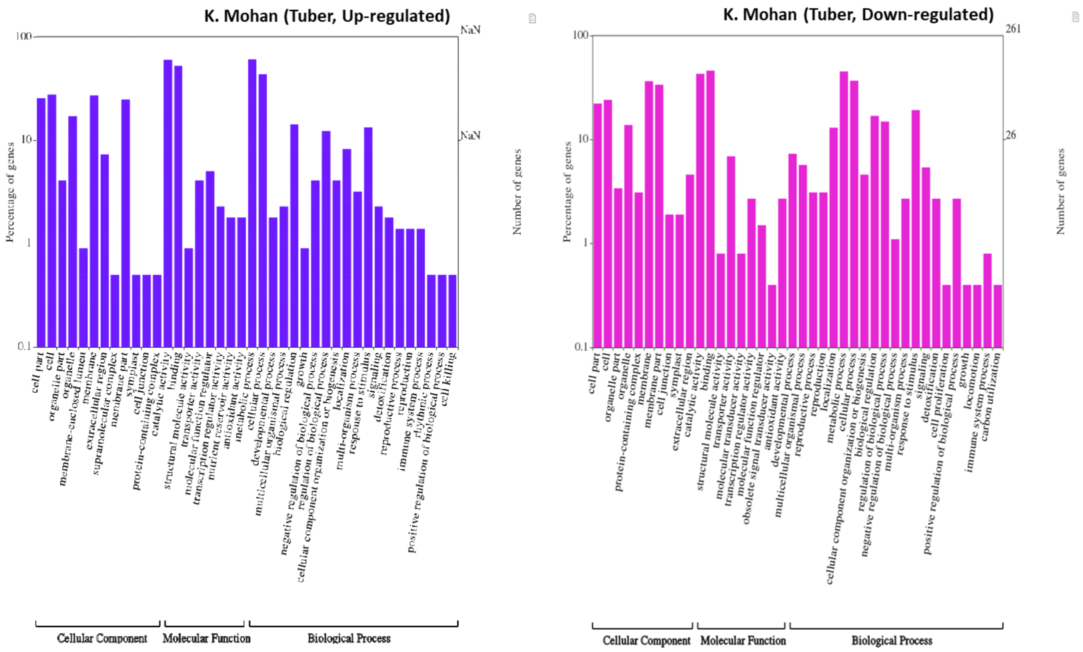

Fig. 6.22. The Gene Ontology (GO) annotation of up-regulated and down-regulated differentially expressed genes (DEGs) in three GO domains (Cellular Component, Molecular Function, and Biological Process) in tuber tissues of Kufri Mohan versus Kufri Sutlej (control).

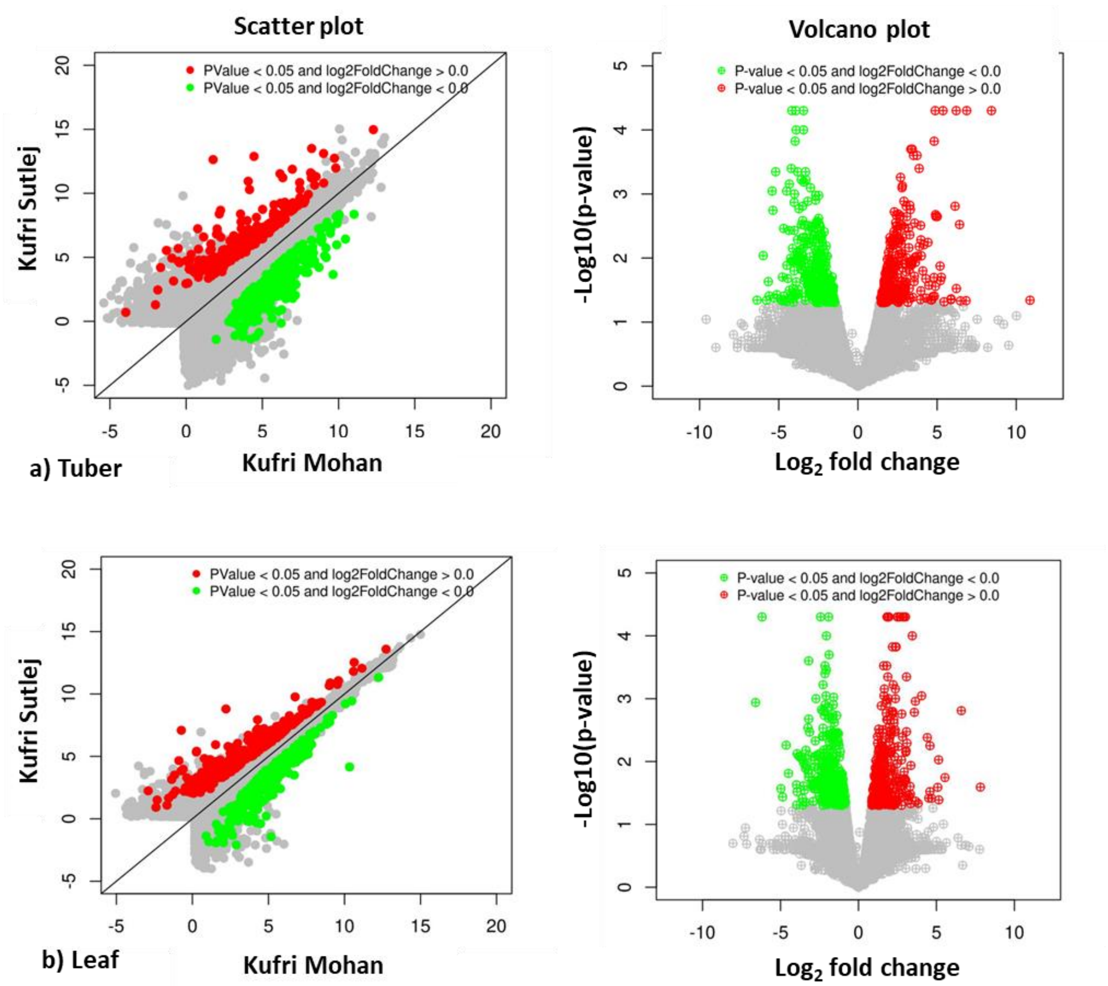

Fig. 6.23. Scatter plot and volcano plots showing significant differentially expressed genes (DEGs) in different colour dots between Kufri Mohan versus Kufri Sutlej (control) in **a)** tuber tissues, **b)** leaf tissues. Dots in different colours represent red (up-regulated), green (down-regulated), and grey (non-significant).

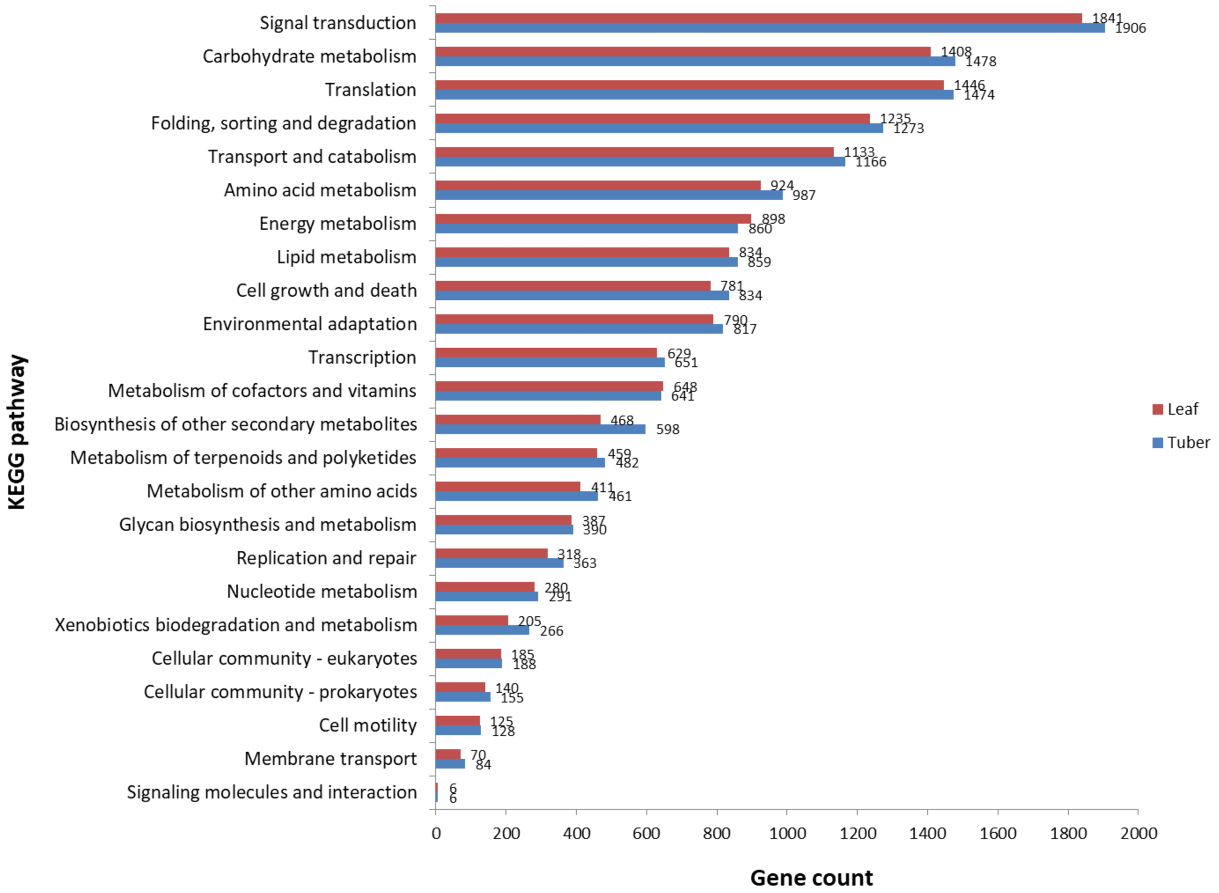

Fig. 6.24. The KEGG pathways classification of annotated genes in leaf and tuber tissues of potato varieties *viz.* Kufri Frysona, Kufri Khyati, Kufri Mohan, and Kufri Sutlej (control) under aeroponics. X and Y axes indicate gene count and KEGG pathways, respectively.

6.4 Discussion

6.4.1 Evaluation of potato varieties under different nitrogen regimes in aeroponics

Reducing the application of nitrogen (N) fertilizers is a practical approach to protect the environment and lower production costs by improving the nitrogen use efficiency (NUE) of plants (Garnett et al., 2015). Plant foliage is essential in influencing photosynthetic efficiency and crop yield per unit area in potatoes, with variations noted depending on N application rates. (Vos, 2009). This study highlighted significant differences in yield and various NUE variables. Several studies indicate that an increased N supply enhances plant biomass by promoting vegetative growth, but when nitrogen is limited, it results in a decrease in leaf area, nitrogen content, and overall yield (Hu et al., 2014). In conditions of N deficiency, N remobilization from the foliage to the tubers occurs early, which accelerates leaf senescence as the plant tries to conserve N for tuber development. This early senescence can limit the plant's photosynthetic capacity and overall growth. Conversely, when N is applied excessively, it can delay plant maturity by prolonging the vegetative phase, which leads to delayed senescence. This extended growth period, however, may reduce the efficiency of nutrient use, ultimately affecting tuber formation and yield (Vos, 2009). Our research reveals notable variation among Indian potato varieties when phenotyped under aeroponic conditions with varied nitrogen levels. This finding aligns with the work of Tiwari et al. (2022), who explored various traits, including root system architecture (RSA), plant biomass, NUE parameters, and yield-related characteristics, which are essential for efficient resource acquisition and improving nitrogen use efficiency (NUE). Plant roots are critical for nutrient and water absorption, which, in turn, affects tuber growth and development in potatoes (Iwama, 2008). The aeroponic experiment provided valuable insights into accurately assessing root system architecture in potatoes, with significant differences observed in RSA traits among varieties, including total root length, surface area, and root volume. The observed RSA traits were significantly and positively correlated with key indicators of plant performance, including biomass accumulation (plant height and leaf area), tuber development (number and yield), and nitrogen use efficiency metrics (AgNUE, NUE, and NUpE), suggesting their potential role in enhancing nutrient acquisition and productivity. Most of the high-yielding varieties demonstrated superior RSA traits compared to poorer-performing varieties. These results are in agreement with previous research that underscores the presence of genetic variation in root traits among potato varieties, suggesting that selection for specific root characteristics could enhance traits like nutrient uptake and overall crop performance. This highlights the potential for breeding programs to target root system architecture as a means of improving yield and nitrogen use efficiency. Consistent with previous findings, root dry weight has demonstrated a positive correlation with final tuber yield (Sattelmacher et al., 1990; Stalham and Allen, 2001; Wishart et al., 2013), underscoring the critical role of root development in yield determination. The functional importance of root traits has been widely recognized across various crop species, particularly in root and tuber crops, where advancements in high-throughput phenotyping have enabled more precise characterization of below-ground architecture and its relationship to nutrient acquisition and productivity (Khan et al., 2016; Li et al., 2016; Paez-Garcia et al., 2015). Potato plants generally have shallow root systems, but the development of deeper basal roots combined with a dense network of shorter roots can significantly enhance nutrient acquisition. This root architecture facilitates better access to water and essential nutrients, particularly in the lower soil layers, which ultimately contributes to improved tuber development and higher overall yield (White et al., 2005). Notably, the basal roots of potatoes serve to anchor the plant and facilitate water uptake, while the stolon roots are responsible for nutrient capture and promoting tuber growth (Villordon et al., 2014). In aeroponics, root growth is unrestricted, leading to a significantly higher root biomass compared to plants grown in the field (Ospina et al., 2014). Therefore, this investigation suggests that optimizing root features is essential for enhancing plant growth and nitrogen use efficiency in potatoes. By improving root architecture, such as increasing root depth and density, breeding programs could potentially boost nitrogen uptake and utilization, leading to higher tuber yields. Additionally, these insights can inform more targeted nutrient management practices, ensuring efficient nitrogen use while minimizing environmental impacts.

Plant phenotypes and yield components influence nitrogen use efficiency (NUE) parameters. Research has shown that higher NUE occurs under limited nitrogen conditions, whereas NUE tends to decrease with increased nitrogen supply in potatoes (Errebhi et al., 1998, 1999; Zebarth et al., 2004). Previous research has identified plant dry biomass and nitrogen uptake as key metrics for evaluating nitrogen use efficiency (NUE) in potato cultivation (Errebhi, 1998; Vos, 1997). Therefore, our study suggests that the key traits for selecting nitrogen-use efficient potato genotypes include NUE variables, plant biomass (both root dry weight and shoot dry weight), and tuber yield. For the majority of the features examined in the aeroponics system with different N conditions, a wide-ranging response was exhibited by the potato cultivars.

6.4.2 Dissecting genes underlying different nitrogen treatments (high and low N) in potato

The potato is a crop that needs a lot of nitrogen fertilizer for a good yield. Thus, it is crucial to breed new cultivars to increase nitrogen use efficiency (NUE) at the plant level while preserving yield. This method will improve soil and air quality, save nitrogen, and lower cultivation costs—all of which are advantageous for the environment and human health. Understanding the genes involved in NUE is vital for future breeding and biotechnological research. The goal of the current study was to look into the genes and regulatory components linked to high tuber production in aeroponic potato plants grown with high nitrogen levels as well as the genes linked to high NUE potential in low nitrogen level conditions. Comparing high nitrogen to low nitrogen environments (control), our findings revealed the presence of particular genes in potato leaves and tuber tissues. In comparison to low nitrogen, we found that high nitrogen levels significantly increased plant biomass and tuber output. Crucially, under low nitrogen conditions, the variety Kufri Pukhraj showed higher NUE than Kufri Jyoti, supporting earlier field research that found Kufri Pukhraj to be a more nitrogen-use-efficient variety than Kufri Jyoti. Our results are in line with previous findings that increased NUE is shown under low nitrogen conditions, whereas high nitrogen increases tuber production and associated characteristics. Prior studies have demonstrated that while low nitrogen produces noticeably smaller potato tubers, high nitrogen encourages the growth of aboveground plant components, including shoots and leaves. Thus, our analysis reveals that although high nitrogen boosts yield, high NUE is attainable under low nitrogen settings. Kufri Pukhraj, the NUE-efficient type, may help preserve nitrogen and safeguard the environment. Aeroponics technology can also be used successfully for a number of studies pertaining to biological growth phases, especially when it comes to comprehending the biology of potatoes' roots, stolons, and tubers.

A popular technique for researching a variety of crops, including potatoes, is transcriptome sequencing. As a result of this method, numerous regulatory molecules linked to various characteristics have been identified. In this work, we discovered several genes that are important for plant stress responses and are probably responsible for high tuber yields and increased nitrogen use efficiency (NUE) in aeroponic potatoes. Notably, we discovered that several hormonal signaling molecules and the glutaredoxin gene play a major role in potatoes' high yields and nitrogen metabolism. Development, stress resistance, redox signaling, hormone control, ion homeostasis, and environmental adaptation are among the vital roles played by the glutaredoxin protein family in plants. These genes are important for potato tuber development and nitrogen metabolism, according to earlier research. The Kufri Pukhraj variety's leaves and tubers showed an interesting upregulation of the glutaredoxin gene, which may have improved nitrogen-use efficiency and increased yields in nitrogen-limited environments. Moreover, it has been demonstrated that overexpression of the CC-type glutaredoxin OsGRX6 affects rice's nitrogen status and hormone signaling. These results are consistent with earlier studies that emphasize the function of glutaredoxin genes in the growth and development of potatoes at different nitrogen concentrations. Plant responses to biotic and abiotic stressors are also significantly influenced by transcription factors (TFs), which are significant regulatory molecules. According to our research, transcription factors like bZIP108, MADS64, and GLK5 play a significant role in nitrogen metabolism. In the tuber tissues of the Kufri Jyoti variety, we found that a protein TF that contains the BTB/POZ domain was elevated. MYB transcription factors, proteins with AP2/ERF domains, and TFs with NAC domains were also found to be differentially regulated across the potato varieties under investigation. Prior research supports the roles of these genes and transcription factors as found in this study, suggesting that they may improve potato yield and nitrogen usage efficiency. Thus, we propose that transcription factors and glutaredoxins are probably important for increasing tuber yields and enhancing NUE in potatoes.

For plants to absorb nutrients, particularly through nitrate transporters in potatoes, the concept of root architecture is essential. The root system architecture (RSA), protein storage, the source-to-sink connection, ionic balance, and reactions to biotic and abiotic stressors are all impacted by these transporters, which are crucial in controlling nitrogen uptake. They also help in the maintenance of the carbon-nitrogen balance. In this investigation, we discovered that the tubers of Kufri Jyoti and Kufri Pukhraj both had overexpressed nitrate transporters and nitrate reductase genes, underscoring their important functions in improving yield and nitrogen metabolism. Our results are in line with earlier studies that highlight the significance of nitrate transporters in plants. Furthermore, this study found ABC transporters, which may have an impact on the pathways for the manufacture of jasmonic acid that control potato tuberization. Water and tiny molecules are transported more easily by the varied family of channel proteins known as aquaporins. They perform vital roles in the transit of metal ions, solutes, and small molecules under biotic and abiotic stresses, making them indispensable for plant development and stress responses. Growing evidence indicates that aquaporins have crucial regulatory functions in several activities, such as fruit ripening, water flow, tissue expansion, seed germination, reproductive growth, and the preservation of plant cellular water homeostasis.

The aquaporin genes TIP1;3 and TIP2;3 were shown to be upregulated in both tuber and leaf tissues in this investigation, indicating their potential role in potato nitrogen metabolism. It has also been demonstrated that the aquaporin gene TIP2;1 functions similarly in other plant species. Glutamate synthetase (GS), a crucial enzyme that transforms inorganic nitrogen into organic forms, is another significant gene group implicated in this process. The two forms of GS found in higher plants are cytosolic (GS1) and plastidic (GS2), with GS2 predominating in the majority of tissues that contain chlorophyll. Aminotransferases are members of the nitrogen metabolism gene group that aid in the production of amino acids and photorespiratory nitrogen absorption. We discovered that the expression of genes involved in nitrogen metabolism, such as glutamine synthetase (GS), asparagine synthetase, and aspartate aminotransferase, varied among our potato cultivars. A prior work has shown that nitrogen supplementation boosts the expression of the gene encoding the peroxisomal enzyme alanine-glyoxylate aminotransferase, which is implicated in the photorespiratory pathway. Glycine and pyruvate are produced from alanine and glyoxylate with the help of this enzyme. All things considered, this study highlights how important the nitrate transporter, aquaporins, GS, and L-asparaginase are to the growth of potato tubers.

The main ingredient in potato tubers is starch, which has drawn increasing attention for both food and non-food uses. In this work, we examined how elevated nitrogen levels affected the expression of genes involved in lipid and sugar metabolism, such as phospholipase, sucrose synthase, sterol desaturase, and GDSL esterase/lipase genes. Sucrose is the major sugar transported in the phloem tissues of most plants following photosynthesis. The glycosyl transferase enzyme sucrose synthase catalyzes the reversible breakdown of sucrose into fructose and either uridine diphosphate glucose or adenosine diphosphate glucose. The glycosyl transferase enzyme ‘sucrose synthase’ catalyzes the reversible breakdown of sucrose into fructose and either uridine or adenosine diphosphate glucose. There has already been discussion of the *GDSL esterase/lipase* gene family's variety and multifunctional significance, especially in rice. In our investigation, we discovered that the Kufri Jyoti variety's tuber tissues had overexpressed sucrose synthase, whereas the leaf tissues had overexpressed glycerophosphodiester phosphodiesterase genes. In contrast, the Kufri Pukhraj variety's leaves showed inhibition of the gene for UDP-glucuronyl/UDP-glucosyl transferase family proteins. Essential enzymes known as UDP-glycosyltransferases (UGTs) help sugars conjugate with tiny lipophilic molecules during plant detoxification and homeostasis processes. These findings are consistent with the previous research, indicating that nitrogen deficiency increases the amount of sugar in the stolons, which promotes tuberization.

Lipid compounds called sterols, which are produced from isoprenoids, are vital components of eukaryotic cells. A complex blend of sterols produced by plants is linked to a number of processes, such as defensive responses and interactions with pathogens. The Kufri Jyoti potato variety's tuber tissues showed an up-regulation of the sterol desaturase gene in this study, suggesting that the gene plays a role in tuber growth and development. Furthermore, phospholipase enzymes are essential to plant metabolism because they catalyze the breakdown of phospholipidsAmong them, phospholipase D is very important in plants because it plays a role in many different processes, such as hormone regulation, stress responses, and plant growth and development. All things considered, earlier research has highlighted the possible functions of genes linked to potato sugar metabolism. As a result, genes involved in the metabolism of sugar and fat are necessary for the growth and development of potato tubers, impacting nitrogen metabolism and yield.

During nitrogen scarcity, genes that respond to stress, heat shock proteins, cell wall proteins, and laccase genes are essential for nitrogen (N) metabolism. In this investigation, we discovered that the Kufri Pukhraj variety's leaves and tubers had higher levels of the stress-responsive gene osmotin, whereas the Kufri Jyoti variety's tubers and leaves had higher levels of the heat shock protein binding protein and proline-rich protein, respectively. Similar stress-related genes that promote the manufacture of jasmonic acid in potatoes under nitrogen stress have also been found in earlier studies. Furthermore, a crucial part of the plant stress response is the cell wall. The xyloglucan endotransglucosylase/hydrolase multigene family controls cell wall reconstruction and helps plants withstand stress. We found that different cell wall proteins in our study play different functions in the production of potato tubers. The xyloglucan endotransglucosylase/hydrolase 1 gene was found to be upregulated in the leaves of the Kufri Pukhraj variety, which was one noteworthy discovery. Additionally, we discovered laccases, which are glycoproteins that contain several copper atoms. These enzymes, which include ferroxidase, ascorbate oxidase, and nitrite reductase, catalyze the oxidation of substances like phenols and acrylamides. Our investigation revealed that the genes for laccase and multicopper oxidase were markedly upregulated in Kufri Pukhraj tubers and leaves, underscoring their critical functions in the growth of potato tubers. According to earlier research, laccases play a role in controlling the polymerization and deposition of lignin in plant cell walls in response to environmental stress. Several overexpressed genes, such as cysteine protease inhibitor 1, miraculin, sterol desaturase, and kunitz-type tuber invertase inhibitor, were found in the tuber tissue of Kufri Pukhraj subjected to low nitrogen (N) stress. Furthermore, we noticed that under low N stress, leaf tissues showed elevated genes such as *transposase, strictosidine synthase*, *pectinesterase, apyrase 3*, *and zinc-binding family protein*. These results imply that stress response genes are essential for potatoes' tuberization and stress tolerance.

6.4.3 Uncovering genes involved in potato tuber growth and development

One of the main phenomena in potatoes is the growth and development of tubers (Ahmad et al., 2022). In addition to traditional field agriculture, potatoes have been grown using aeroponics technology all over the world to produce high-quality seed tubers quickly (Tunio et al., 2020). This research assessed the root morphology, phenotypic, and tuber yield-linked characteristics of several potato types grown aeroponically. In comparison to control Kufri Sutlej, Kufri Mohan is the highest yielding variety, followed by Kufri Frysona and Kufri Khyati, based on the observations recorded on plant biomass, foliage, and tuber yield components. Furthermore, Kufri Frysona and Kufri Mohan both have the longest roots. Prior research on the agronomic performance of potato types grown in aeroponics (Buckseth et al., 2016; Tiwari et al., 2020) was in accordance with our findings. Furthermore, Kufri Frysona and Kufri Mohan both have the longest total roots. Prior research on the agronomic performance of potato types grown in aeroponics (Buckseth et al., 2016; Tiwari et al., 2020) was in line with our research. Also, our results demonstrated variation in the root morphology traits in the potato varieties, which is in line with their conclusions. Aeroponics technology seems to have several potential qualities that could make it an effective system for producing potatoes, according to Čížek and Komárková (2022). To evaluate high-yielding potato varieties at an early stage, aeroponics is a viable method.to support future seed research, biotechnology, and rapid breeding.

To uncover the fundamental genes accountable for tuber yield and its contributing features of potato grown in an aeroponic system, RNA sequencing has been used. Consequently, genes like *lipoxygenase*, *101 kDa heat shock protein, cysteine protease inhibitor 1*, *Kunitz-type tuber invertase inhibitor*, *laccase*, and *catechol oxidase B chloroplastic* were found to be markedly overexpressed in the tuber tissues of Kufri Mohan, Kufri Frysona, and Kufri Khyati. According to an investigation, the aforementioned genes are linked to a range of responses to biotic and abiotic stress tolerance and features that contribute to potato yield (Tiwari et al., 2020). Potato tuber formation is regulated by the lipoxygenase gene (Kolomiets et al., 2001). According to Zhang et al. (2024), new genes may play a role in tuber formation, which could impact potato productivity and quality. Throughout tuber development and stolon initiation, these genes control various activities, including blooming, metabolism and signal transduction, cell division, sucrose transport, and starch and hormone production. These genes' functional roles in several metabolic pathways are demonstrated by their *in silico* characterization for conserved motifs and the InterPro database. In this study, gene expression markers were developed to identify promising high-yielding genotypes at the seedling stage using an aeroponics cultivation system. Below is a brief overview of possible genes connected to tuber yield-related characteristics.

Genes taking part in the metabolism of carbohydrates or sugars are important for the growth and development of potato tubers. This study found several genes, including *UGT gene family 1 protein, glucosyltransferase, UDP-glucose glucosyltransferase, fatty acyl-CoA reductase 3* and *lipoxygenase,* that may be involved in the metabolism of carbohydrates. Their function in the formation and development of potato tubers is supported by extensive research. For example, the UGT genes are essential for glycosylation and the plant's response to abiotic stress (Guan et al., 2024). Cotton leaf senescence has been examined in relation to the UGT gene family (Chen et al., 2022). Second, there are functionally varied classes of dioxygenases called plant lipoxygenases (LOXs) that are linked to physiological responses like stress, senescence, and growth. According to Kolomiets et al. (2001), the *LOX1* class genes are said to control the development of potato tubers. Additionally, tuber development is determined by genes linked to stress. While *GDSL esterase/lipase*, *glutathion S-transferase (GST)*, *dehydration-responsive protein RD22*, and *stress-associated protein 3* all demonstrated critical functions, the gene *101 kDa heat shock protein* was significantly up-regulated in tuber tissues amongst these three types. The growth, development, and stress response of plants are significantly influenced by the GDSL esterase/lipase. In 2021, Zhang et al. looked into the gene family of *GDSL-type esterase/lipase* genome-wide identification in wild wheat (*Dasypyrum villosum*). Ain-Ali et al. (2021) have validated the function of the dehydration-responsive element binding gene family in potatoes. In plants, GST has a variety of roles, such as stress tolerance and cellular detoxification. According to a thorough genome-wide investigation of gene families of 90 GST, potatoes respond to biotic and abiotic stress in a variety of ways (Islam et al., 2018). The glycosyl transferase enzyme sucrose synthase carries out the enzymatic breakdown of sucrose into fructose and nucleotide diphosphate glucose, which is a reversible reaction. According to Baroja-Fernández et al. (2009), the study shows that the upregulated expression of sucrose synthase led to a significant escalation in the potato's overall yield, tuber dry weight, starch, UDPglucose, and ADPglucose content. Numerous pieces of evidence thus show that *stress-associated proteins, LOX, heat shock proteins, UDP-GTs/UGTs, GST, GDSL esterase/lipase*, *X*, and other genes related to the metabolism of sugars are involved in the creation of potato tubers. In potatoes and other higher plants, the sucrose transporter plays a critical role in plant growth and development by mediating the loading of sucrose phloem of source tissue and its unloading into its sink tissue (Gong et al., 2023). The development, flowering time, and tuber production of potato plants are influenced by the sucrose transporter gene StSUT2 (Gong et al., 2023). All things considered, our research offers information on the metabolism of carbohydrates and stress-responsive genes that influence tuber growth and the characteristics associated with yield in potatoes.

In potatoes, transcription factors are essential for controlling gene expression. Several transcription factors were allegedly linked to tuber yield and its component features in potatoes in the current investigation. Transcription factors that were recognized are as follows: *WRKY TF 6, bHLH63, basic helix-loop-helix protein bHLH7, MYB, R2R3-MYB, zinc finger family protein, Myb-like transcription factors, R18, MYB1-2, white-brown-complex ABC transporter family, and BURP domain-containing protein*. Former research reveals the significance of transcription factors in the growth and development of the potato. For instance, the analysis of MADS-box genes in potato indicates that *StMADS1* and *StMADS13* are potential subsequent markers of the tuberigen gene *StSP6A*, suggesting their involvement in tuber development regulation (Gao et al., 2018). Moreover, the function of transcription factors such as *bHLH* and *bZIP* in abiotic stress tolerance has been proven by several studies (Guo et al., 2021; Wang et al., 2021). A prominent gene family with considerable functional importance involved in controlling the growth and development of plants, as well as abiotic stress response, is the MYB family (Sun et al., 2019). According to our research, transcription factors are prerequisites for controlling transcription during the growth of potato tubers, which in turn affects yield and other characteristics. Therefore, our research clarifies the possible transcription factors that might be involved in tuber yield in aeroponics.

In potatoes, the phytohormones—primarily GA and auxin—are crucial for the beginning of stolons and the formation of tubers. Important DEGs were found in this investigation, including GA20 oxidase, auxin-induced protein X10A, and auxin-induced protein 5NG4. Changes in the expression of the GA 20-oxidase gene are known to have a significant impact on the tuber induction and production of the potato plant (Carrera et al., 2000). Under controlled circumstances, researchers have shown the function of several tuber-inducing genes in potatoes (Mahajan et al., 2016). Significantly, auxins are essential for both tuber development and resistance (Kolachevskaya et al., 2019). Another study revealed that elevated auxin concentrations, namely indole acetic acid (IAA), in transgenic tubers compared to non-transgenic or control tubers favour potato-tuberization (Kolachevskaya et al., 2015). Astounding development has been reported in identifying genes governing the mechanism of formation of tubers in potato. According to recent research, a number of genes, including *StBEL5 (BEL1-LIKE TF)*, *StMSI1*, and *POTATO HOMEOBOX 15 TF*, in conjunction with epigenetic and photoperiod mechanisms of gene expression, control tuber growth in potatoes (Kondhare et al., 2021). We also found that these genes were expressed differently in this study, which is consistent with these findings.

Cysteine protease inhibitor 1, BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1, laccase, catechol oxidase B chloroplastic, phosphoethanolamine N-methyltransferase, Kunitz-type tuber invertase inhibitor, globulin, phenylalanine ammonia-lyase 1, and a few other genes have also been found to play an important part in the development of potato tuber, which contributes to yield component traits. According to earlier research, the BAK1 is a flexible kinase that influences ABA-induced signaling in plants in a positive way and is implicated in a wide range of developmental responses (Shang et al., 2022). Additionally, plant enzymes called laccases play critical roles in plant growth and lignin polymerization, and environmental stress-induced development (Hashemipetroudi et al., 2023). The primary protein found in potatoes is called patatin or tuberin, which is made up of various protease inhibitors and is a fraction of albumin and globulin (Peksa and Miedzianka, 2021). This study discovered a wide range of DEGs that play a significant part in tuber yield in potatoes. As a result, our research identified the possible contribution of transcription factors, kinases, and other genes to tuber yield and its associated features in potatoes.

CHAPTER 7
Conclusion and Future Remarks

CHAPTER 7 CONCLUSION AND FUTURE REMARKS

This study provides insights into the variation among Indian potato varieties regarding agronomic traits and Nitrogen Use Efficiency (NUE) under different nitrogen regimes (high and low N) input in aeroponic conditions. Findings of this study revealed a diverse range of responses in varieties for plant biomass, root morphology, yield-contributing traits, and NUE variables. This diversity was consistent across old to new varieties (year of release: 1960s to 2020), and highlights the use of diverse sources in breeding programs. Overall, popular and high-yielding varieties demonstrated improved performance in terms of RSA, plant biomass, tuber yield, and NUE. The conclusion generated from this study is valuable for the development of new potato varieties. Additionally, evaluating potato varieties under different nitrogen regimes (low and high nitrogen) in aeroponics as well as field conditions provides information for low-input agricultural systems.

According to this study, Kufri Jyoti shows a high yield in aeroponics under high nitrogen settings, while the Kufri Pukhraj variety is nitrogen-use efficient under low nitrogen conditions. Several candidate genes that are probably involved in producing high tuber yields in aeroponics under high nitrogen levels have been found by transcriptomic profiling. These genes include a nitrate transporter, glutamine synthetase, aminotransferase, GDSL esterase/lipase, sucrose synthase, UDP-glycosyltransferases, osmotin, xyloglucan endotransglucosylase/hydrolase, laccases, glutaredoxin, and several transcription factors (like BTB/POZ, AP2/ERF, and MYB). These genes may be linked to managing nitrogen stress in aeroponics under different nitrogen conditions and good tuber output in the potato cultivars Kufri Jyoti and Kufri Pukhraj. Genes that are probably involved in the potato plant's above-ground (leaf) and below-ground (tuber) portions are shown schematically in Figure 6.12. This comprises both up-regulated and down-regulated genes in the Kufri Pukhraj variety under high nitrogen levels for the optimal tuber output in aeroponics. To functionally describe the putative genes linked to high yield and NUE in plants and to corroborate these findings with field-level results, further study is required. All things considered, this work offers important new information on the possible genes influencing tuber yield and NUE in potatoes grown in aeroponics under high versus low nitrogen conditions.

Significant phenotypic changes between potato types grown aeroponically were also found in this investigation. Additionally, RNA-seq results identified possible target genes related to tuber yield-related features in potatoes, including genes related to carbohydrate metabolism (glucosyltransferase, *UDP-GT*), stress-response (*GST*, *GDSL* esterase/lipase, dehydration-responsive protein *RD22*, and stress-associated protein 3), transcription factors (*MYB*, *R2R3-MYB*, *R18*, *MYB1-2*, white-brown-complex ABC transporter family, *WRKY TF 6*, *bHLH63*, *BHLH7,* and *BURP* domain-containing protein), phytohormoes (auxin-induced protein *X10A*, auxin-induced protein *5NG4*, and *GA20* oxidase), kinase proteins (cysteine protease inhibitor 1, Kunitz-type tuber invertase inhibitor, BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1), and other genes (laccase, and catechol oxidase B chloroplastic). The functional annotation and characterization of these genes contribute to a deeper understanding of motif identification, InterPro domain assignments, and sequence-based phylogenetic relationships. RNA-seq results were validated by using real-time PCR analysis to validate specific genes. Using gene expression markers, high-yielding cultivars were chosen early on in the aeroponics process. Functional characterisation of specific genes will be necessary in the future. All things considered, our research sheds light on genes that may be important in identifying tuber yield and its constituent characteristics in potatoes grown in an aeroponic environment.

Bibliography

- Abelenda JA, Cruz-Oro E, Franco-Zorrilla, JM, Prat S (2016) Potato *StCONSTANS*-like1 suppresses storage organ formation by directly activating the FT-like *StSP5G* repressor. Curr Biol 26:872–881. https://doi.org/10.1016/j.cub.2016.01.066
- Afzal Z, Howton TC, Sun Y, Mukhtar MS. The roles of aquaporins in plant stress responses. J. Dev. Biol. 2016; 4:9. https://doi.org/10.3390/jdb4010009
- Ahmad D, Zhang Z, Rasheed H, Xu X, Bao J (2022) Recent advances in molecular improvement for potato tuber traits. Int J Mol Sci 23:9982. https://doi.org/10.3390/ijms23179982
- Ahn S-J, Marygold S J. The UDP-glycosyltransferase family in drosophila melanogaster: nomenclature update, gene expression and phylogenetic analysis. Front. Physiol. 2021; 12:648481. https://doi.org/10.3389/fphys.2021.648481
- Ain-Ali QU, Mushtaq N, Amir R, Gul A, Tahir M, Munir F (2021) Genome-wide promoter analysis, homology modeling and protein interaction network of dehydration responsive element binding (DREB) gene family in *Solanum tuberosum*. PLoS One 16(12):e0261215. https://doi.org/10.1371/journal.pone.0261215
- Anderson JM, Boardman NK. Studies on the greening of dark grown bean plants. II. Development of photochemical activity. Australian J Biol Sci. 1964; 17:93–101
- Arsenault J-L, Poulcur S, Messier C, Guay R (1995) WinRHIZOTM, a root measuring system with a unique overlap correction method. HortScience 30(4):906D–906
- Badr, M.A., Ali, E. & Salman, S.R. Effect of Nitrogen Application and Fertigation Scheduling On Potato Yield Performance Under Drip Irrigation System. c 75, 2909–2918 (2023). https://doi.org/10.1007/s10343-023-00871-y.
- Bailey T L, Elkan C (1994) Fitting a mixture model by expectation maximization to discover motifs in biopolymers. Procd Inter Conf Intel Syst Mol Biol 2:28–36

- Baroja-Fernández E, Muñoz FJ, Montero M, Etxeberria E, Sesma MT, Ovecka M, Bahaji A, Ezquer I, Li J, Prat S, Pozueta-Romero J (2009) Enhancing sucrose synthase activity in transgenic potato (*Solanum tuberosum* L.) tubers results in increased levels of starch, ADPglucose and UDPglucose and total yield. Plant Cell Physiol 50(9):1651-1662. https://doi.org/10.1093/pcp/pcp108.
- Buckseth T, Tiwari J K, Singh R K, Singh A K, Challam C, Zinta R, Sharma S, Sharma A K, Sadawarti M, Kumar M, Kumar V. Transcriptome analysis reveals molecular signatures associated with Apical Rooted Cutting (ARC) Technology in seed potato production. J Plant Growth Regul. 2024; 43: 1042-1060. https://doi.org/10.1007/s00344-023-11158-x.
- Buckseth, T., Sharma, A.K., Pandey, K.K., Singh, B.P., Muthuraj, R. (2016). Methods of pre-basic seed potato production with special reference to aeroponics - a review. *Scientia Horticulturae* 204: 79–87.
- Buckseth, T., Singh, R. K., Tiwari, J. K., Sharma, A. K., Singh, S., and Chakrabarti, S. K. (2020). A novel sustainable aeroponic system for healthy seed production in India-an update. Indian J. Agric. Sci. 90 (2), 243–248.
- Buckseth, T., Singh, R. K., Tiwari, J. K., Sharma, S., Gautam, A., Sharma, A. K., et al. (2022). Influence of microplant hardening methods on aeroponic potato minituber production. Potato. Res 65, 335-348. doi: 10.1007/s11540-021-09530-z.
- Cabianca A, Ruthes AC, Pawlowski K, Dahlin P. Tomato sterol 22-desaturase gene CYP710A11: its roles in *Meloidogyne incognita* infection and plant stigmasterol alteration. Int. J. Mol. Sci. 2022; 23:15111. https://doi.org/10.3390/ijms232315111.
- Carrera E, Bou J, Garcia-Martinez JL, Prat S (2000) Changes in GA 20-oxidase gene expression strongly affect stem length, tuber induction and tuber yield of potato plants. The Plant J 22: 247-256. https://doi.org/10.1046/j.1365-313x.2000.00736.x.
- Chakrabarti SK, Conghua X, Tiwari JK (2017) The Potato Genome. Springer International Publishing AG 2017. Springer Nature, Switzerland. pp. 326. https://doi.org/10.1007/978-3-319-66135-3.

- Chapman HD, Pratt PF. Methods of analysis for soils, plants and waters. University of California, Berkeley, CA, USA; 1961; 150.
- Chen Y, Fu M, Li H, Wang L, Liu R, Liu Z (2022). Genome-wide characterization of the UDP-glycosyltransferase gene family reveals their potential roles in leaf senescence in cotton. Int J Biol Macromol 222 (Pt B): 2648-2660. https://doi.org/10.1016/j.ijbiomac.2022.10.047.
- Chepyshko H, Lai C P, Huang L M, Liu J H, Shaw J F. Multifunctionality and diversity of GDSL esterase/lipase gene family in rice (*Oryza sativa* L. *japonica*) genome: new insights from bioinformatics analysis. BMC Genomics 2012; 13:309. https://doi.org/10.1186/1471-2164-13-309.
- Čížek M, Komárková Z (2022). Comparison of aeroponics technology with a conventional system of production of potato minitubers in the conditions of the Czech Republic. Plant Soil Environ 68 (8): 366–374. https://doi.org/10.17221/164/2022-PSE.
- Ding K, Shan Y, Wang L, Zhang Y, Tian G. Transcriptomics combined with physiological analysis and metabolomics revealed the response of potato tuber formation to nitrogen. BMC Plant Biol. 2024; 24(1), 1109. https://doi.org/10.1186/s12870-024-05758-2.
- Duque, L.O., Villordon, A. (2019). Root branching and nutrient efficiency: status and way forward in root and tuber crops. *Frontiers in Plant Science* 10: 237.
- El-Kereamy A, Bi YM, Mahmood K, Ranathunge K, Yaish MW, Nambara E, Rothstein SJ. Overexpression of the CC-type glutaredoxin, OsGRX6 affects hormone and nitrogen status in rice plants. Front Plant Sci. 2015; 6:934. https://doi.org/10.3389/fpls.2015.00934.
- Errebhi, M., Rosen, C.J., Lauer, F.I., Martin, M.W., Bamberg, J.B., Birong, D.E. (1998). Screening of exotic potato germplasm for nitrogen uptake and biomass production. *American Journal of Potato Research* 75: 93–100.
- Errebhi, M., Rosen, C.J., Lauer, F.I., Martin, M.W., Bamberg, J.B. (1999). Evaluation of tuber-bearing Solanum species for nitrogen use efficiency and biomass partitioning. *American Journal of Potato Research* 76: 143–151.

- Fageria NK, Baligar, VC, Li YC. The Role of nutrient efficient plants in improving crop yields in the twenty first century. J Plant Nutr. 2008; 31:1121–1157. https://doi.org/10.1080/01904160802116068.
- Fan X, Naz M, Fan X, Xuan W, Miller AJ, Xu G. Plant nitrate transporters: from gene function to application. J Exp Bot. 2017; 68:2463–2475. https://doi.org/10.1093/jxb/erx011.
- Fukushima A, Kusano M (2014) A network perspective on nitrogen metabolism from model to crop plants using integrated 'omics' approaches. Journal of Experimental Botany 65, 5619–5630. doi:10.1093/jxb/eru322.
- Gálvez JH, Tai HH, Lagüe M, Zebarth BJ, Strömvik MV. The nitrogen responsive transcriptome in potato (*Solanum tuberosum* L.) reveals significant gene regulatory motifs. Sci Rep. 2016; 6:26090. https://doi.org/10.1038/srep26090.
- Gao H, Wang Z, Li S, Hou M, Zhou Y, Zhao Y, Li G, Zhao H, Ma H (2018) Genome-wide survey of potato MADS-box genes reveals that *StMADS1* and *StMADS13* are putative downstream targets of tuberigen *StSP6A*. BMC Genomics 19(1):726. https://doi.org/10.1186/s12864-018-5113-z.
- Garnett, T., Conn, V., Kaiser, B.N. (2009). Root based approaches to improving nitrogen use efficiency in plants. *Plant Cell and Environment* 32: 1272–1283.
- Garnett, T., Plett, D., Heuer, S., Okamoto, M. (2015). Genetic approaches to enhancing nitrogen-use efficiency (NUE) in cereals: challenges and future directions. *Functional Plant Biology* 42: 921–941.
- Goffart, J.P., Olivier, M., Frankinet, M. (2008). Potato crop nitrogen status assessment to improve N fertilization management and efficiency: past-present-future. *Potato Research* 51: 355–383.
- Gong HL, Liu JB, Igiraneza C, Dusengemungu L (2023). Sucrose transporter *StSUT2* affects potato plants growth, flowering time, and tuber yield. Curr Issues Mol Biol 45(3):2629-2643. https://doi.org/10.3390/cimb45030172.
- Guan H, Zhang Y, Li J, Zhu Z, Chang J, Bakari A, Chen S, Zheng K, Cao S (2024). Analysis of the UDP-Glucosyltransferase (*UGT*) gene family and its functional involvement in drought and salt stress tolerance in *Phoebe bournei*.

Plants (Basel) 13(5):722. https://doi.org/10.3390/plants13050722.

- Guo H, Pu X, Jia H, Zhou Y, Ye G, Yang Y, Na T, Wang J. Transcriptome analysis reveals multiple effects of nitrogen accumulation and metabolism in the roots, shoots, and leaves of potato (*Solanum tuberosum* L.). BMC Plant Biol. 2022; 22(1), 282. https://doi.org/10.1186/s12870-022-03652-3.
- Guo J, Sun B, He H, Zhang Y, Tian H, Wang B (2021) Current understanding of bHLH transcription factors in plant abiotic stress tolerance. Int J Mol Sci 22(9):4921. https://doi.org/10.3390/ijms22094921.
- Gutiérrez, R.A. Systems biology for enhanced plant nitrogen nutrition Science, 336 (2012), pp. 1673-1675.
- Hashemipetroudi SH, Arab M, Heidari P, Kuhlmann M (2023) Genome-wide analysis of the laccase (LAC) gene family in *Aeluropus littoralis*: a focus on identification, evolution and expression patterns in response to abiotic stresses and ABA treatment. Front Plant Sci 14:1112354. https://doi.org/10.3389/fpls.2023.1112354.
- Howe EA, Sinha R, Schlauch D, Quackenbush J (2011) RNA-Seq analysis in MeV. Bioinformatics 27(22): 3209–3210. https://doi.org/10.1093/bioinformatics/btr490.
- https://opstat.somee.com/ Online Statistical Analysis Tool (OPSTAT). Accessed on 08th January 2025.
- Hu, W., Sun, Z.P., LI, T.L., Yan, H.Z., Zhang, H. (2014). Nitrogen nutrition index and its relationship with N use efficiency, tuber yield, radiation use efficiency, and leaf parameters in potatoes. *Journal of Integrative Agriculture* 13: 1008–1016.
- Islam MS, Choudhury M, Majlish AK, Islam T, Ghosh A (2018). Comprehensive genome-wide analysis of *Glutathione S-transferase* gene family in potato (*Solanum tuberosum* L.) and their expression profiling in various anatomical tissues and perturbation conditions. Gene 639:149-162. https://doi.org/10.1016/j.gene.2017.10.007.
- Iwama, K. (2008). Physiology of the potato: new insights into root system and repercussions for crop management. *Potato Research* 51: 333–353.

- Jiang L, Ball G, Hodgman C, Coules A, Zhao H, Lu C. Analysis of gene regulatory networks of maize in response to nitrogen. Genes 2018; 9:151. https://doi.org/10.3390/genes9030151.
- Joshi M, Fogelman E, Belausov E, Ginzberg I (2016) Potato root system development and factors that determine its architecture. J Plant Physiol 205:113-123. https://doi.org/10.1016/j.jplph.2016.08.014.
- Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M (2016) KEGG as a reference resource for gene and protein annotation. Nucl Acids Res 44(D1):D457-462. https://doi.org/10.1093/nar/gkv1070.
- Khan, M.A., Gemenet, D.C., Villordon, A. (2016). Root system architecture and abiotic stress tolerance: current knowledge in root and tuber crops. *Frontiers in Plant Science* 7: 1584.
- Koch, M., Naumann, M., Pawelzik, E., Gransee, A., Thiel, H. (2020). The Importanceof nutrient management for potato production part I: plant nutrition and yield. *Potato Research* 63: 97–119.
- Kolachevskaya OO, Alekseeva VV, Sergeeva LI, Rukavtsova EB, Getman IA, Vreugdenhil D, Buryanov YI, Romanov GA (2015) Expression of auxin synthesis gene tms1 under control of tuberspecific promoter enhances potato tuberization in vitro. J Integr Plant Biol 57: 734–744. https://doi.org/10.1111/jipb.12314.
- Kolachevskaya OO, Lomin SN, Arkhipov DV, Romanov GA (2019) Auxins in potato: molecular aspects and emerging roles in tuber formation and stress resistance. Plant Cell Rep 38:681–698. https://doi.org/10.1007/s00299-019-02395-0.
- Kolomiets MV, Hannapel DJ, Chen H, Tymeson M, Gladon RJ (2001) Lipoxygenase is involved in the control of potato tuber development. Plant Cell 13(3):613-626. https://doi.org/10.1105/tpc.13.3.613.
- Kondhare KR, Kumar A, Patil NS, Malankar NN, Saha K, Banerjee AK (2021) Development of aerial and belowground tubers in potato is governed by photoperiod and epigenetic mechanism. Plant Physiol 187(3):1071-1086. https://doi.org/10.1093/plphys/kiab409.

- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7):1870-1874. https://doi.org/10.1093/molbev/msw054.
- Lehretz GG, Sonnewald S, Hornyik C, Corral JM, Sonnewald U (2019) Post-transcriptional regulation of *FLOWERING LOCUS T* modulates heat-dependent source-sink development in potato. Curr Biol 29: 1614–1624.
- Li L, Zhang C, Zhang M, Yang C, Bao Y, Wang D, Chen Y, Chen Q. Genome-wide analysis and expression profiling of the phospholipase D gene family in *Solanum tuberosum*. Biology 2021; 10:741. https://doi.org/10.3390/biology10080741.
- Li, X., Zeng, R., Liao, H. (2016). Improving crop nutrient efficiency through root architecture modifications. *Journal of Integrative Plant Biology* 58: 193–202.
- Lindahl V, Gourdon P, Andersson M, Hess B. Permeability and ammonia selectivity in aquaporin TIP2;1: linking structure to function. Sci. Rep. 2018; 8:2995. https://doi.org/10.1038/s41598-018-21357-2.
- Liu, A. Transcriptomic reprogramming in soybean seedlings under salt stress. *Plant Cell Environ.* 42, 98–114 (2019).
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using realtime quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262.
- Lu, Y., Zhang, J., Han, Z., Han, Z., Li, S., Zhang, J., Ma, H., & Han, Y. Screening of differentially expressed microRNAs and target genes in two potato varieties under nitrogen stress. BMC Plant Biol. 2022; 22(1), 478. https://doi.org/10.1186/s12870-022-03866-5.
- Luthra SK, Gupta VK, Tiwari JK, Kumar V, Bhardwaj V, Sood S, Dalamu, Kaur RP, Kumar R, Vanishree G, Kumar D, Mhatre P, Chakrabarti SK (2020) Potato breeding in India. Tech Bull No. 74 (Revised), ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India. pp. 1-214.
- Lynch, J.P. (2019). Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture. *New Phytologist* 223: 548–

564.

- Mahajan AS, Kondhare KR, Rajabhoj MP, Kumar A, Ghate T, Ravindran N, Habib F, Siddappa S, Banerjee AK (2016) Regulation, overexpression, and target gene identification of *Potato Homeobox 15* (*POTH15*) - a class-I *KNOX* gene in potato. J Exp Bot 67(14):4255-4272. https://doi.org/10.1093/jxb/erw205.
- Morris WL, Hancock RD, Ducreux LJ, Morris JA, Usman M, Verrall SR, Sharma SK, Bryan G, McNicol JW, Hedley PE, Taylor MA (2014) Day length dependent restructuring of the leaf transcriptome and metabolome in potato genotypes with contrasting tuberization phenotypes. Plant Cell Environ 37:1351–1363. https://doi.org/10.1111/pce.12238.
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.
- Muthamilarasan M, Bonthala VS, Khandelwal R, Jaishankar J, Shweta S, Nawaz K, Prasad M. Global analysis of WRKY transcription factor super family in Setaria identifies potential candidates involved in abiotic stress signaling. Front. Plant Sci. 2015; 6:910. https://doi.org/10.3389/fpls.2015.00910.
- Oliveros JC (2007-2015) Venny, an interactive tool for comparing lists with Venn's diagrams. https://bioinfogp.cnb.csic.es/tools/venny/index.html.
- Ospina, C.A., Van Bueren, E.T.L., Allefs, J.J.H.M., Engel, B., van der Putten, P.E.L., van der Linden C.G., Struik, P. C. (2014). Diversity of crop development traits and nitrogen use efficiency among potato cultivars grown under contrasting nitrogen regimes. *Euphytica* 199: 13–29.
- Paez-Garcia, A., Motes, C.M., Scheible, W.-R., Chen, R., Blancaflor, E.B., Monteros, M.J. (2015). Root traits and phenotyping strategies for plant improvement. *Plants* (Basel) 4: 334–355.
- Parenteau M T, Gu H, Zebarth B J, Cambouris A N, Lafond J, Nelson A, Nyiraneza J, Davidson C, Lagüe M, Galvez J H et al. Data mining nitrogen-responsive gene expression for source–sink relations and indicators of N status in potato. Agronomy 2020; 10:1617.

https://doi.org/10.3390/agronomy10101617.

- Peksa A, Miedzianka J (2021) Potato industry by-products as a source of protein with beneficial nutritional, functional, health-promoting and antimicrobial properties. Appl Sci 11:3497. https://doi.org/10.3390/app11083497.
- Plantenga FDM, Bergonzi S, Abelenda JA, Bachem CWB, Visser RGF, Heuvelink E, Marcelis LFM (2019). The tuberization signal *StSP6A* represses flower bud development in potato. J Exp Bot 70:937–948. https://doi.org/10.1093/jxb/ery420.
- Potato Genome Sequencing Consortium (2011). Genome sequence and analysis of the tuber crop potato. Nature 475:189–195. https://doi.org/10.1038/nature10158.
- Rubio-Pifia JA, Zapata-Peter O. Isolation of total RNA from tissues rich in polyphenols and polysaccharides of mangrove plants. Electron J Biotechnol. 2011; https://doi.org/10.2225/vol14-issue5-fulltext-10.
- Sattelmacher, B., Kuene, R., Malagamba, P., Moreno, U. (1990). Evaluation of tuber bearing Solanum species belonging to different ploidy levels for its yielding potential at low soil fertility. *Plant and Soil* 129: 227–233.
- Schum, A., Jansen, G. (2014). *In vitro* method for early evaluation of nitrogen use efficiency associated traits in potato. *Journal of Applied Botany and Food Quality* 87: 256 – 264.
- Shang Y, Yang D, Ha Y, Hur YS, Lee MM, Nam KH (2022) Brassinosteroid-insensitive 1-associated receptor kinase 1 modulates abscisic acid signaling by inducing PYR1 monomerization and association with ABI1 in *Arabidopsis*. Front Plant Sci 13:849467. https://doi.org/10.3389/fpls.2022.849467.
- Sharifi, M., Zebarth, B.J., Coleman, W. (2007). Screening for nitrogen-use efficiency in potato with a recirculating hydroponic system. *Communications in Soil Science and Plant Analysis* 38: 359–370.
- Sheoran, O.P., Tonk, D.S., Kaushik, L.S., Hasija, R.C., Pannu, R.S. (1998). Statistical software package for agricultural research workers. In: Hooda DS, Hasija RC (eds.) Recent advances in information theory, statistics & computer applications. Department of Mathematics Statistics, CCS HAU, Hisar. p 139-

143.

- Shrestha, B., Darapuneni, M., Stringam, B. L., Lombard, K., & Djaman, K. (2023). Irrigation Water and Nitrogen Fertilizer Management in Potato (Solanum tuberosum L.): A Review. Agronomy, 13(10), 2566. MDPI AG. http://dx.doi.org/10.3390/agronomy13102566.
- Simons M, Saha R, Guillard L, Clément G, Armengaud P, Cañas R, Maranas CD,Lea PJ, Hirel B (2014). Nitrogen-use efficiency in maize (Zea mays L.): from‘omics’studies to metabolic modelling. Journal of Experimental Botany 65, 5657–5671. doi:10.1093/jxb/eru227.
- Singh AK, Janakiram T, Chakrabarti SK, Bhardwaj V, Tiwari JK. Indian Potato Varieties. ICAR-CPRI, Shimla, 2018; ISBN: 978-93-5361-215-3. Pages: 179.
- Singh, D., Chhonkar, P.K., Dwivedi, B.S. (2005). Manual of soil, plant and water analysis. Westville Publishing House, New Delhi, pp. 200.
- Stalham, M.A., Allen, E.J. (2001). Effect of variety, irrigation regime and planting date on depth, rate, duration and density of root growth in the potato (*Solanum tuberosum*) crop. *The Journal of Agricultural Science* 137: 251–270.
- Sun W, Ma Z, Chen H, Liu M. (2019). MYB gene family in potato (*Solanum tuberosum* L.): genome-wide identification of hormone-responsive reveals their potential functions in growth and development. Int J Mol Sci 20:4847. https://doi.org/10.3390/ijms20194847.
- Tiwari JK, Buckseth B, Singh RK, Zinta R, Thakur K, Bhardwaj V, Dua VK, Kumar M. Aeroponic evaluation identifies variation in Indian potato varieties for root morphology, nitrogen use efficiency parameters and yield traits. J Plant Nutr. 2022; 45:2696-2709. https://doi.org/10.1080/01904167.2022.2046080.
- Tiwari JK, Buckseth T, Singh RK, Kumar M, Kant S. Prospects of improving nitrogen use efficiency in potato: lessons from transgenics to genome editing strategies in plants. Front. Plant Sci. 2020; 11:597481. https://doi.org/10.3389/fpls.2020.597481.
- Tiwari JK, Buckseth T, Zinta R, Saraswati A, Singh RK, Rawat S, Dua VK, Chakrabarti SK (2020) Transcriptome analysis of potato shoots, roots and stolons under nitrogen stress. Sci Rep 10:1152. https://doi.org/10.1038/s41598-

020-58167-4.

- Tiwari, J. K., Singh, R. K., Buckseth, T., and Chakrabarti, S. K. (2019). Aeroponics: A novel precision system to study nutrient biology in potato. Indian Horti. 64 (5), 33–34.
- Tiwari, J.K., Buckseth, T., Devi, S., Varshney, S., Sahu, S., Patil, V.U., Zinta, R., Ali,N., Moudgil, V., Singh, R.K., Rawat, S., Dua, V.K., Kumar, D., Kumar, M., Chakrabarti, S.K., Rao, A.R., Rai, A. (2020a): Physiological and genome-wide RNA- sequencing analyses identify candidate genes in a nitrogen-use efficient potato cv. Kufri Gaurav. *Plant Physiology and Biochemistry* 154: 171-183.
- Tiwari, J.K., Buckseth, T., Zinta, R., et al., Genome-wide identification and characterization of microRNAs by small RNA sequencing for low nitrogen stress in potato. PLoS ONE, 2020, 15(5):e0233076.
- Tiwari, J.K., Devi, S., Buckesth, T., Ali, N., Singh, R.K., Zinta, R., Dua, V.K., Chakrabarti, S.K. (2020b). Precision phenotyping of contrasting potato (*Solanum tuberosum* L.) varieties in a novel aeroponics system for improving nitrogen use efficiency: in search of key traits and genes. *Journal of Integrative Agriculture* 19: 51-61.
- Tiwari, J.K., Plett, D., Garnett, T., Chakrabarti, S.K., Singh, R.K. (2018). Integrated genomics, physiology and breeding approaches for improving nitrogen use efficiency in potato: translating knowledge from other crops. *Functional Plant Biology* 45: 587–605.
- Tiwari, JK, Buckseth, T, Singh, RK, Zinta, R, Thakur, K, Bhardwaj, V, Dua, V.K., & Kumar, M. Aeroponic evaluation identifies variation in Indian potato varieties for root morphology, nitrogen use efficiency parameters, and yield traits. Journal of Plant Nutrition, 2022. DOI: 10.1080/01904167.2022.2046080.
- Tracy, S.R., Nagel, K.A., Postma, J.A., Fassbender, H., Wasson, A., Watt, M. (2020). Crop improvement from phenotyping roots: highlights reveal expanding opportunities. *Trends Plant Science* 25: 105–118.
- Trapnell C, Hendrickson DG, Sauvageau M, Goff L, Rinn JL, Pachter L. Differential analysis of gene regulation at transcript resolution with RNA-seq.

Nature Biotechnol. 2013; 31:46. https://doi.org/10.1038/nbt.2450.

- Trapnell C, Hendrickson DG, Sauvageau M, Goff L, Rinn JL, Pachter L (2013) Differential analysis of gene regulation at transcript resolution with RNA-seq. Nat Biotechnol 31:46–53. https://doi.org/10.1038/nbt.2450.
- Trapnell C, Pachter L, Salzberg S L. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 2009; 25:1105–1111. https://doi.org/10.1093/bioinformatics/btp120.
- Trapnell C, Pachter L, Salzberg SL (2009) TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25:1105–1111. https://doi.org/10.1093/bioinformatics/btp120.
- Trehan, S.P., Singh, B.P. (2013). Nutrient efficiency of different crop species and potato varieties - in retrospect and prospect. *Potato Journal* 40: 1–21.
- Tunio MH, Gao J, Shaikh SA, Lakhiar IA, Qureshi WA, Solangi KA, Chandio FA (2020). Potato production in aeroponics: an emerging food growing system in sustainable agriculture for food security. Chilean J Agric Res 80(1): 118-132. https://doi.org/10.4067/S0718-58392020000100118.
- Van Bueren, E.T.L., Struik, P.C. (2017). Diverse concepts of breeding for nitrogen use efficiency- a review. *Agronomy for Sustainable Development* 37: 50.
- Van Harsselaar J K, Lorenz J, Senning M, Sonnewald U, Sonnewald S. Genome-wide analysis of starch metabolism genes in potato (*Solanum tuberosum* L.). BMC Genomics 2017; 18:37. https://doi.org/10.1186/s12864-016-3381-z.
- Villordon, A.Q., Ginzberg, I., Firon, N. (2014). Root architecture and root and tuber crop productivity. *Trends in Plant Science* 19: 419–425.
- Vos J. The nitrogen response of potato (*Solanum tuberosum* L.) in the field: nitrogen uptake and yield, harvest index and nitrogen concentration. Potato Res. 1997; 40: 237–248. https://doi.org/10.1007/BF02358249.
- Vos, J. (2009). Nitrogen responses and nitrogen management in potato. *Potato Research* 52: 305–317.

- Wang Q, Guo C, Li Z, Sun J, Wang D, Xu L, Li X, Guo Y (2021) Identification and analysis of bZIP family genes in potato and their potential roles in stress responses. Front Plant Sci 12:637343. https://doi.org/10.3389/fpls.2021.637343.
- Wang, C., Zang, H., Liu, J., Shi, X., Li, S., Chen F., Chu, Q. (2020). Optimum nitrogen rate to maintain sustainable potato production and improve nitrogen use efficiency at a regional scale in China: a meta-analysis. *Agronomy for Sustainable Development* 40: 37.
- Wei Q, Yin Y, Tong Q, Gong Z, Shi Y. Multi-omics analysis of excessive nitrogen fertilizer application: Assessing environmental damage and solutions in potato farming. Ecotoxicol. Environ. Saf. 2024; 284, 116916. https://doi.org/10.1016/j.ecoenv.2024.116916.
- Westennann, D. T. (2005). Nutritional Requirements of Potatoes. Amer J of Potato Res (2005) 82:301-307.
- White, P.J. (2019). Root traits benefitting crop production in environments with limited water and nutrient availability. *Annals of Botany* 124: 883–890.
- White, P.J., Broadley, M.R., Hammond, J.P., Thompson, A.J. (2005). Optimising the potato root system for phosphorus and water acquisition in low-input growing systems. *Aspects of Applied Biology* 73: 111–118.
- Wishart J., George, T.S., Brown, L.K., Ramsay, G., Bradshaw, J.E., White, P.J., Gregory, P.J. (2013). Measuring variation in potato roots in both field and glasshouse: the search for useful yield predictors and a simple screen for root traits. *Plant and Soil* 368: 231–249.
- Wu Q, Yang J, Cheng N, Hirschi K D, White F F, Park S. Glutaredoxins in plant development, abiotic stress response, and iron homeostasis: from model organisms to crops. Environ Exp Bot. 2017; 139:91–98. https://doi.org/10.1016/j.envexpbot.2017.04.007.
- Ye J, Zhang Y, Cui H, Liu J, Wu Y, Cheng Y, Xu H, Huang X, Li S, Zhou A, Zhang X, Bolund L, Chen Q, Wang J, Yang H, Fang L, Shi C (2018) WEGO 2.0: a web tool for analyzing and plotting GO annotations, 2018 update. Nucl Acids Res 46(W1): W71-W75. https://doi.org/10.1093/nar/gky400.

- Yi X, Sun X, Tian R, Li K, Ni M, Ying J, Xu L, Liu L, Wang Y. Genome-wide characterization of the aquaporin gene family in radish and functional analysis of *RsPIP2-6* involved in salt stress. Front. Plant Sci. 2022; 13:860742. https://doi.org/10.3389/fpls.2022.860742.
- Zebarth, B.J., Tai, G., Tarn, R., de Jong, H., Milburn, P.H. (2004). Nitrogen use efficiency characteristics of commercial potato cultivars. *Canadian Journal of Plant Science* 84: 589–598.
- Zebarth, B.J., Tarn, T.R., de Jong, H., Murphy, A. (2008). Nitrogen use efficiency characteristics of andigena and diploid potato selections. *American Journal of Potato Research* 85: 210–218.
- Zhang H, Zhang X, Zhao J, Sun L, Wang H, Zhu Y, Xiao J, Wang X (2021) Genome-wide identification of GDSL-type esterase/lipase gene family in *Dasypyrum villosum* L. reveals that *DvGELP53* is related to BSMV infection. Int J Mol Sci 22:12317. https://doi.org/10.3390/ijms222212317.
- Zhang J, Han Z, Lu Y, Zhao Y, Wang Y (2021). Genome-wide identification, structural and gene expression analysis of the nitrate transporters (*NRTs*) family in potato (Solanum tuberosum L.). PLoS ONE 16(10): e0257383.
- Zhang J, Han Z, Lu Y, Zhao Y, Wang Y, Zhang J, Ma H, Han YZ. Genome-wide identification, structural and gene expression analysis of the nitrate transporters (NRTs) family in potato (*Solanum tuberosum* L.). PloS One 2021; 16(10), e0257383. https://doi.org/10.1371/journal.pone.0257383.
- Zhang J, Wang Y, Zhao Y, Zhang Y, Zhang J, Ma H, Han Y. Transcriptome analysis reveals Nitrogen deficiency induced alterations in leaf and root of three cultivars of potato (*Solanum tuberosum* L.). PloS One 2020; 15(10), e0240662. https://doi.org/10.1371/journal.pone.0240662.
- Zhang J, Wang Y, Zhao Y, Zhang Y, Zhang J, Ma H, Han, Y. (2020). Transcriptomeanalysis reveals Nitrogen deficiency induced alterations in leaf and root of three cultivars of potato (Solanum tuberosum L.). PLoS ONE 15(10): e0240662.
- Zhang J-Z, He P-W, Xu X-M, Lü Z-F, Cui P, George MS, Lu G-Q. Genome-wide identification and expression analysis of the xyloglucan

endotransglucosylase/hydrolase gene family in sweet potato [*Ipomoea batatas* (L.) Lam]. Int. J. Mol. Sci. 2023; 24:775. https://doi.org/10.3390/ijms24010775.

- Zhang M, Jian H, Shang L, Wang K, Wen S, Li Z, Liu R, Jia L, Huang Z, Lyu D (2024) Transcriptome analysis reveals novel genes potentially involved in tuberization in potato. Plants (Basel) 13(6):795. https://doi.org/10.3390/plants13060795.
- Zhang, J., Han, Z., Lu, Y., Zhao, Y., Wang, Y., Zhang, J., Ma, H., Han, Y.Z. Genome-wide identification, structural and gene expression analysis of the nitrate transporters (NRTs) family in potato (Solanum tuberosum L.) PLoS ONE 2021, 16(10):e0257383.
- Zhang, J., Wang, Y., Zhao, Y., Zhang, Y., Zhang, J., Ma, H., Han, Y. Transcriptome analysis reveals Nitrogen deficiency induced alterations in leaf and root of three cultivars of potato (Solanum tuberosum L.) PLoS ONE 2020, 16(6): e0253994.
- Zinta R, Tiwari JK, Buckseth T, Goutam U, Singh RK, Thakur AK, et al. (2025) Agro-physiological and transcriptome profiling reveal key genes associated with potato tuberization under different nitrogen regimes in aeroponics. PLoS ONE 20(3): e0320313. https://doi.org/10.1371/journal.pone.0320313.
- Zinta R, Tiwari JK, Buckseth T, Thakur K, Goutam U, Kumar D, Challam C, Bhatia N, Poonia AK, Naik S, Singh RK, Thakur AK, Dalamu D, Luthra SK, Kumar V and Kumar M (2022). Root system architecture for abiotic stress tolerance in potato: Lessons from plants. Front. Plant Sci. 13:926214.doi: 10.3389/fpls.2022.9262.
- Zinta, R., Tiwari, J. K., Buckseth, T., Goutam, U., Singh, R. K., Kumar, V., & Thakur, A. K. (2024a). Transcriptome profiling and characterization of genes associated with tuberization under high temperature in aeroponics in potato cv. Kufri Anand. Genes & genomics, 46(4), 409–421. https://doi.org/10.1007/s13258-024-01503-z.
- Zinta, R., Tiwari, J.K., Buckseth, T., Goutam, U., et al. (2024b) Phenotypic and transcriptomics characterization uncovers genes underlying tuber yield traits and gene expression marker development in potato under aeroponics. Planta 260, 74 (2024). https://doi.org/10.1007/s00425-024-04507-x.

- Zinta R, Tiwari JK, Buckseth T, Goutam U, Singh RK, Thakur AK, et al. (2025) Agro-physiological and transcriptome profiling reveal key genes associated with potato tuberization under different nitrogen regimes in aeroponics. PLoS ONE 20(3): e0320313. https://doi.org/10.1371/journal.pone.0320313.