The effect of potato nucleus tuber size on plant development and seed yield

Thesis submitted in the partial fulfilment of requirements for the degree of

MASTER OF PHILOSOPHY

In

Plant Genomics and Biotechnology

By

Muhammad Arif

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PARC Institute of Advanced Studies in Agriculture

National Agricultural Research Centre, Islamabad

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Certificate

The thesis submitted by **Muhammad Arif** to Plant Genomics and Biotechnology (PGB), PARC Institute of Advanced Studies in Agriculture (PIASA), National Agriculture Research Centre (NARC), Quaid-I-Azam University, Islamabad, Pakistan, is accepted in its current form. This thesis fulfills the entire requirement for facilitating him with Degree of Master of Philosophy in **Plant Genomics and Biotechnology**.

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AUTHOR'S DECLARATION

I would like to divulge that the data presented in this thesis "**In-vitro Multiplication and Effect f Tuber Size on Potato Seed Production**" is generated by myself from original research work under the supervision of **Dr. Aish Muhammad** at Department of Plant Genomics and Biotechnology (PGB), PARC Institute of Advanced Studies in Agriculture (PIASA), National Agriculture Research Centre (NARC), Quaid-I-Azam University Islamabad, Pakistan.

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Muhammad Arif

DEDICATION

I would like to dedicate my research work to my loving and caring parents, **Mr. and Mrs. Rash Khan** whose prays, and support made my path more comfortable. They are my mentor, and I am proud of them**.**

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List of Abbreviations

1. COV	Coefficient of variance
2. ANOVA	Analysis of variance
3. RCBD	Randomized Complete Block Design
4. DF	Degree of freedom
5. NS	Non-significan
6. IAA	Indole acetic acid
7. **KH_2PO_4**	Potassium diphosphate
8. LSD	Least Significant Difference
9. GA3	Gibberellic acid
10. MS	Murashige and Skoog
11. NARC	National Agricultural Research Center
12. NIGAB	National Institute of Genomics and Advanced Biotechnology
13. pH	power of hydrogen

ABSTRACT

Potatoes (*Solanum tuberosum* L.), the world's most dominant tuber crop, play an important role in maintaining human nutrition and food security. The objectives of experiment were invitro multiplication of different potato varieties and optimization of tuber size for multiplication in field under different environmental conditions. The study material, mini tubers were collected from tissue culture Lab NIGAB, NARC and field experiment was conducted at two different locations of Gilgit i.e Babusar and Naltar during the cropping season of 2020-21. Research was conducted at two different locations during the growing season of 2020-21 at the farms of Agricultural Research Gilgit. The experiment was conducted to study the in-vitro multiplication and effect of tuber size on potato seed production. Six potato nucleus tuber sizes were sown in the first week of June using Randomized Complete Block Design (RCBD) with 03 replications at different locations with plant to plant 1 ft and row to row 2.5 ft distance. Each treatment consisted of 90 tubers, 30 tubers in each block and each block contain 3 rows 10 tubers in each. For accurate results uniform cultural practices were carried out for various treatments in each replication. Plants were irrigated as per requirements. Data was recorded from 10 randomly selected plants for each treatment in each replication. Morphological parameters consist of days to germination percentage (%), first germination, days to physiological maturity, days to flowering, leaves per plant, plant height (cm), number of main stems, tuber weight (g), tubers per plant, tuber yield (t/ha), unmarketable tuber yield (t/ha), marketable tuber yield (t/ha), weight of large tuber (g), weight of small tubers (g). Physiological parameters were consisting of total starch yield (t/h), specific gravity tuber starch content (g/100g), and tuber dry matter content. Data of the studied traits were analyzed by using computer statistical package, Statistix 8.1, to calculate the genetic variability for different traits of potato treatments. Least significant differences (LSD) test was also applied. ANOVA showed significant differences for the treatments for days to first germination, days to flowering, germination percentage, days to physiological maturity, leaves per plant, number of main stems per plant, plant height, tubers per plant, tuber yield, marketable tuber yield, size of large tubers, tuber starch content, total starch yield. Environment showed significant difference for days to first germination, germination percentage, days to flowering, days to physiological maturity, number of leaves per plant, number of main stems per plant, tuber weight, marketable tuber yield, size of large tuber, tuber starch content, total starch yield, while treatment and environment interaction showed non-significant differences for marketable tuber yield. The present results showed that tuber size affected yield, implying that plants from larger tubers performed well compared to smaller seeds. Large tubers contain a larger number of buds, resulting in a large number of stems and thus a large number of branches and leaves, while small tubers have a smaller number of leaves. The number of leaf yields was maximum on plants from very large tubers, decreasing almost evenly when going from large, medium to small tubers.

INTRODUCTION

Potatoes (*Solanum tuberosum* L.), the world's most dominant tuber crop, play an important role in maintaining human nutrition and food security (Karim *et al.,* 2010). It has shallow rooting system and heavy nutrients requirements. There is a high requirement of this crop because of high growth rate and short growing period (Durr and Lorenzl, 1981).

Potatoes are member of the Solanaceae family, are grown and eaten in a variety of countries (FAO, 2016). Potatoes (*Solanum tuberosum* L.) are the fourth most produced food after wheat, maize, and rice (FAOSTAT, 2020). It is highly recommended for eating because of the energy characteristics obtained from carbs (Fernandes et al., 2015). In 2018, the globe produced about 368 million tons of potatoes on 17.5 million hectares (FAOSTAT, 2020). The International Potato Center (CIP) and its collaborators have shown that potatoes play a dual function in food security, first as a cash commodity on the market and then as a food crop with high nutritional content (Devaux et al. 2014). According to FAO statistics, Asia produces the most potatoes, followed by Europe, South America, and North and Central America (FAOSTAT Agriculture, 2012).

The potato (*Solanum tuberosum* L.) is a member of the Solanaceae family and is cultivated globally. Pakistan's potato output is much lower than that of industrialized nations. The potato (*Solanum tuberosum L.*) is a major vegetable and the world's primary cash crop. It is an annual herbaceous crop with a short growing season that provides a high caloric yield in a short period of time. The edible portion of the plant is the tuber, which develops at the end of subterranean stems known as stolon. It is a low-cost food source, an industrial raw material, an animal feed source, and a seed tuber. It is the fourth most widely grown food crop, after wheat, rice, and maize, and the most widely cultivated dicotyledonous crop (Khatun et al., 2003 and Abdel Alleam, 2015). It is a nutritious food that is high in carbohydrates, vitamins, minerals, and protein.

Asia produces the most potatoes, followed by Europe, South America, and finally North and Central America (FAO, 2012). It is also a significant crop in Pakistan, with 159.4 thousand hectares under cultivation and an output of 3491.7 thousand tons, yielding an average of 18.5

tons per hectare in 2010-11 (MINFAL, 2011). Potatoes generate a remarkable amount of edible energy and protein per unit area and time than many other crops (Kaur et al., 2015). Potatoes are usually propagated via vegetative means. Pathogen contamination of seed material (bacteria, viruses, and fungus) results in a significant decrease in yield. As a result, despite enormous efforts, no progress has been made in the traditional seed plant potato production system.

Low multiplication rate, loss of a significant proportion of food material, lack of homogeneity, exposure to infectious diseases/pests, and progressive aggregation of degenerative viruses are all characteristics of traditional potato seed production methods (Kaur et al., 2015).

The main cause of substantial yield loss in potatoes is viral illness. By using tissue culture to create disease-free clones, farmers may improve their output and revenue. The absence of healthy and certified seeds is the main cause of the decrease in potato production in Pakistan. Low-yielding cultivars, erroneous agronomic choices, poor management methods, and inefficient land use increase the problem (FAO, 2009; Qasim et al., 2013). Low multiplication rate, loss of a significant proportion of food material, lack of homogeneity, exposure to infectious diseases/pests, and progressive aggregation of degenerative viruses are all characteristics of traditional potato seed production methods (Kaur et al., 2015).

Haberlandt, (1902) developed the idea of cell culture and was the first to try cultivating isolated plant cells *invitro* on an artificial medium. Furthermore, *invitro* micro propagation may be utilized to preserve, store, and distribute potato germplasm (Chaudhary and Mitta, 2014). Tissue culture is a method that involves aseptically growing cells, tissues, or organ pieces of a given plant in artificial culture media in a controlled environment with the goal of developing new plants (Morais et al., 2018). This technique is distinguished by its quickness and high rate of multiplication in a short period of time (Mohapatra & Batra, 2017).

Tissue culture is a great way to obtain homogeneous, pathogen-free microtubers for a healthy, robust crop with higher yields. Potato tuberization is influenced by both genetic (Madhu et al., 2014) and environmental (Kittipadukal et al., 2012) factors, as well as sucrose content. A farmer's traditional method of propagation is tuber propagation. There are two main drawbacks to traditional clonal replication of potato seed stocks. To begin with, there is a poor multiplication rate in the field, which may take up to 12 years, resulting in a lack of flexibility in response to changing end-user requirements. Second, potatoes have a high vulnerability to viral, bacterial, and fungal infections. Micro propagation is becoming a viable alternative to traditional potato propagation.

Tissue culturing is a quick and easy solution to a variety of issues. The mass production of microtubers and the generation of virus-free seed potatoes are two of the many advantages of using invitro techniques (Hoque 2010, Rocha et al, 2015.; Wang and Hu,1982.; Islam and Chowdhury,1998; Khan et al 2003). Microtuber production is simple, inexpensive, and can be done at any time of the year (Rolot, 2012). Microtubers also cut the time it takes to make seed tubers and the number of field generations needed to produce higher-quality seed tubers (Prematilake and Mendis,1999). Microtubers are very simple to carry and store (Hoque, 2010).

Tissue culture is a method of growing new plants by aseptically cultivating cells, tissues, or organ pieces of a given plant in artificial culture media in a controlled environment (Morais et al., 2018). This technique is distinguished by its quickness and high rate of multiplication in a short period of time (Mohapatra & Batra, 2017).

Micropropagation is a technique for producing disease-free seed potatoes that is widely used. This method has two stages: 1) invitro multiplication and plantlet generation, and 2) greenhouse minituber development. We can improve the production of potatoes and other crops by upgrading the tissue culture method. Biotechnology has the potential to help solve these issues while also benefiting potato growers. Plant regeneration from cell and tissue culture is an important part of biotechnology because it has the ability to enhance current cultivars as well as generate new ones in a shorter time frame than traditional breeding methods (Abdelaleem, 2015). Since de-differentiation and the subsequent organogenesis/embryogenesis with the accompanying genetic changes have been reported (Anoop and Chauhan J, 2009. Seed tuber physiological quality and health had an impact on yield (Momena et al., 2014). It's a high-value crop with a higher yield and more nutrients per unit area per unit time than any other major crop Traditional tubers have a poor multiplication rate and are susceptible to diseases (Badoni A and Chauhan J, 2010).

Vegetative growth via tubers is the hallmark of commercial potato production. As a result, the propagation material must be replaced on a regular basis since it may degrade due to cumulative contamination by viruses, fungal, and bacterial illnesses (Al Hussaini et al., 2015; Pereira & Fortes, 2003). *Invitro* tuberization is the process of growing tiny tubers in a laboratory setting. Seed tubers, on the other hand, often expose plants to diseases, particularly viral illnesses (Abraham Dieme et al., 2013). The use of micro tubers has a number of benefits. In contrast to traditional propagation, which results in viral transfer to subsequent generations, meristem culture *invitro* offers a repeatable and economically feasible technique for generating pathogen-free plants.

A well-established method known as micropropagation or plant tissue culture, has been employed often in the horticulture sector to achieve fast multiplication of plants and homogenous material (Sabir et al., 2014). A single explant may be multiplied into thousands of plants in a short period of time by employing this method. Several commercial labs have been established all across the globe in a very short period of time since the 1980s in order to harness the potential of micropropagation for mass production of clonal plants. Improvement of micropropagation protocols necessitates a methodical approach that begins with a thorough understanding of the plant species or genotype's in vivo propagation characteristics and continues with the optimization of various chemical, physical, and environmental factors for growth and multiplication in *invitro* culture (Ruffoni & Savona, 2013).

Tissue culture has also been used to improve potato output via micropropagation, pathogen-free propagule growth, and germplasm preservation, taking into consideration the significance of potato clones (Bhuiyan, 2013). Potato tissue culture regeneration helps with a variety of tasks, including micropropagation of rare plants, hybridization potency maintenance, variation conservation and application, and genetic transformation (Cingel et al., 2010). One of the most significant issues is the high cost of tuber seed production, as well as the lengthy reproductive cycle, which raises the risk of viral and bacterial infections (Van et al., 2012). To address the issue, using tissue culture technology to increase output while lowering production costs by avoiding illnesses and pesticide usage is a viable option (Alison et al., 2011). Application of various growth regulators is thought to alter the chemical makeup of potatoes

(Zahoor & Faheem, 2014). Another recognised factor affecting *invitro* organogenesis is that callus induction and regeneration capacity are largely genotype dependent (Rani et al., 2013). The fast production of callus in many potato cultivars is a highly significant biotechnological research goal (Sabeti et al., 2013).

Micro-propagation is the most successful technique for propagating potatoes, and it is a viable alternative to traditional methods. In terms of genetic and physiological uniformity, it has been shown to be a highly effective method for speeding up the generation of high-grade disease-free plantlets (Sathish et al., 2011). Plantlets may be transplanted from invitro to in vivo settings via microtuberization (Wang and Hu, 1982). Many nations utilise microtubers to cultivate disease-free potatoes (Wang and Hu 1982, Chaudhary and Mittal, 2014). Microtuberization of potatoes requires the proper combination of many variables, including relatively low temperatures (15-20^{o} C) and high sucrose and cytokinin concentrations (Wang and Hu, 1982, Al-Hussaini et al. 2015).

The introduction of microtubers and minitubers into seed production has revolutionised potato production, allowing for a shorter field cycle to produce enough seed potatoes while still ensuring a high degree of base material physical condition (Wróbel, 2014). Microtubers are the first generation of nuclear seed potatoes, weighing between 24 and 273 mg each (Ranalli, 2007). Micro-tubers are little seed potatoes that provide a bridge between invitro plantlets and mini-tubers, allowing delicate vegetative plantlets to be transplanted from invitro to in vivo to generate mini-tubers (Nistor et al., 2010; Liljana et al., 2012; Husain et al., 2017). By following the check testing of material at the early stage, micro-tuber may be a primary source of disease-free seed to complement or even replace the conventional way of potato breeder seed production (Somani and Venkatasalam, 2012). As a result, micro-tuber production is expected to transform global potato output (Kanwal and Shoaib, 2006; Dessoky et al., 2016).

Mini-tubers (Sharma et al., 2008; Altindal and Karadogan, 2010; Nistor et al., 2010; Wróbel, 2014; Srivastava et al., 2015) and micro-tubers (Sharma et al., 2008; Altindal and Karadogan, 2010; Nistor et al., 2010; Wróbel, 2014; Srivastava A well-established technique for the generation of potato mini-tubers is direct in vivo seeding of micro-plants in a soil medium beneath aphid resistant nets/polyhouses (Kumar et al., 2011, Sharma and Pandey, 2013, 2014 and 2017). Minitubers are generated from plantlets or microtubers in general (Saha et al., 2013). Tubers are an important nutritional source of carbohydrates, protein, antioxidants, and vitamins, and they assist the plant as a storage organ as well as a vegetative propagation mechanism throughout the world (Barrell et al., 2013).

Minituber production through plantlets is a simple, low-cost method that has been described as a traditional minituber production system (Dimante and Gaile, 2014), as well as a minituber production programme utilising Invitro plantlets. Plant regeneration from cell and tissue culture is an important part of biotechnology because it can enhance current cultivars as well as generate new ones in a shorter time frame than traditional breeding methods (Abdelaleem, 2015).

Its productivity is influenced by several factors i.e high nutrients requirement especially nitrogen, varieties, sowing period, environmental and geographical conditions (Arsenault *et al.,* 2001). As plant population enhances, observed the reduction in plant parameters and its productivity. This is because of the competition of plants on the availability of water, nutrients, and sunlight. That's why it's required to understanding about the interaction among individual plants with environment. This will lead us to optimize the crop productivity according to ideal crop density level.

Baritelle et al., (1999) demonstrated that the small tubers are less sensitive than the larger tubers. This is because of the high kinetic energy of large tubers. The prime objective of their work was to evaluate the effect of potatoes tuber size on potato tuber properties. A higher grasp of tuber measurement impacts these residences will assist in deciding future selection techniques, via removing tuber measurement as a confounding factor. Potatoes cultivars weighing between 113 to 454g indicating dimension stages many times encountered in managing conditions have been used. Samples from the stem ends of these tubers had been examined to failure the use of dynamic axial compression (strain fee = 80s1) at a temperature of 8 C. The consequences exhibit that whilst tubers over 340 g are substantially much less difficult than those below 100g and 70g while tubers between 70g, 100g and 340g do no longer have drastically unique failure residences (failure stress, failure stress and shock wave speed). Thus, tuber sampling for assessments of such residences can use tubers in the 170 to 340 g vary barring considerable results of tuber size.

Michael et al., (2012) reported that the medium spacing level will give higher productivity as compared to low and high spacing level. They performed an experiment in which three spacing system were established i.e 90 by 15 cm, 90 by 30 cm and 90 by 45 cm. the result showed that the highest yield was achieved by the medium spacing level (90 by 30 cm) as compared to other ones (90 by 15 cm and 90 by 45 cm).

Barani et al., (2013) conducted the experiment to examine the impact of seed length and sprouting of potato (*Solanum tuberosum*). They look at modified into laid finished in factorial layout (3 × 3) the usage of Marfona, Agria and Draga cultivars and three levels of GA3 (10, 5 and 0 mg/l). Consequences indicated that software program of GA3 at low concentrations (five and 10 mg/lit) changed into able to increase widespread performance and productiveness of seed tubers of potatoes. Seed tuber production turned into accelerated through software of the use of GA3 in all cultivars. General weight of seed tubers produced via utility of 5 mg/lit GA3 turn out to be statistically specific as compared to govern. Additionally, outcomes confirmed that after one week from software program of GA3, starch content material reduced, and overall content of sugar extended in potato tuber. Moreover, reported that sugar content is one of the critical traits to identifying the sprouting of potatoes.

Bussan et al., (2007) estimated the impact of plant and tuber set, potato yield, tuber length distribution, and special best elements. Average tuber length turned into related to stem, the usage of the inverse yield law and expected most common tuber length of >200 g. The distribution for tuber sizes turns out to be predicted as a weibull hazard density feature that anticipated modifications in tuber period in reaction to stem and tuber density. Modeling tuber length distribution over different location presents a mechanism for future economic analysis to optimize manipulate and conduct sensitivity analysis to decide the maximum vital factors influencing crop cost.

Zebenay, (2015) reported that the increase in planting density will enhances the total tuber numbers per unit area. The highest tuber growth can be achieved by closer row spacing while the intra row spacing will leads to the reduction in plant population. This is because of the employment of soil minerals and other growth parameters. It also has increased growth due to high densities of plant which covered the young leaves and stops its growth that's why tuber growths is starting earlier and are of good qualities.

Mostofa et al., (2019) demonstrated the effect of size of tuber on the productivity and quality on potatoes. They used five cultivars with size of 10g (T1), 20g (T2), 30g (T3), 40g (T4) and 50g (T5) respectively. Among these cultivars, the tuber size greater than 40g showed the maximum determination (44.35 N), specific gravity (1.08 g/cm^{-3}), dry matter (22.77%), flesh color (L*- 75.60; a*- 11.76; b*- 24.96). The quality of the potatoes reduces with the increased in stored period and become non-reliable for both table and processing means. That's why the commercial farmers may use large sized tubers for good quality product processing.

Potatoes contain maximum proteins, vitamins, mineral and trace element contents. It has relatively high protein content per unit area of land as compared to other crops (Paul, 1985). According to current scenario, increased population level and food security become hot topic not only among the scientists but also for farmers. This is due to high demand of food, high starvation rate and farmer socio-economical condition. This crop is dominating among other crops as it is demanded by all socio-economical classes. Its demand is increasing day by day with respect to elevation of standard of living especially in developing countries. As regarding the good productivity, commercial farmers that are wealthy can grow Irish potato variety as it needs high input production costs. The seed cost is the highest proportion of total production costs (Karim *et al.,* 2010). Accatino and Malagamba (1982) evaluated that the sowing of seed potatoes tubers needs 40-70% of crop production costs in developing countries. The 50% production costs represent the seed tuber costs was reported in Egypt (El Bedewy *et al.,* 1994). The optimum yield and good economical returns of potatoes are achieved by the proper management of seed size and placement level in a specific topographic place (Kabir *et al.,* 2004). The leaf and tuber growth both are correlated and is necessary for the estimation of ideal tuber size, leads to high productivity of potatoes. These parameters are highly depending upon the purpose of growth whether it is for seed or ware potatoes production. In Zimbabwe, four cultivars of potatoes were grown which has four different sized seed i.e from small to very large but seeding rate depends on the weight of about 2 tons per ha of potatoes tubers. Therefore, it is needed to ensure optimum productivity by establishment of proper seeding rate, seeding size and placement spacing. For proper growth and development of potatoes and its productivity, seed to seed distance is maintained which also depend on the cultivars (Zamil *et al.,* 2010). Regarding high level of research on potatoes declared that the plant population and tuber size are highly interlinked with growth and productivity. Various research was carried out few years ago (Bremner and Taha, 1966).

The search for plant populations is by no means old-fashioned due to the unique tuber qualities of modern genotypes and the changing tuber needs of developing industries (Wurr *et al.,* 1993). There is, however, much to explore, even beyond the simple interrelationships between bulbous trees and herbs and the interference between branches. An understanding of these relationships must allow the crop to provide a variety of responses, such as radiation interception and influence on local weather patterns at maturity, prior to tuber formation and management of the wide variety and size of tubers at maturity (Kooman *et al.,* 1996). Study was once aimed at discovering out how potato seed tubers of exceptional sizes engage at various space ranges to impact pre-emergence rate, haulm boom at a range of tuber increases stages.

Aims and Objectives

- Invitro multiplication of different potato varieties.
- Optimization of tuber size for multiplication in field under different environmental conditions.

MATERIALS AND METHODS

2.1 Experimental Material and Sites

The experimental material, mini tubers were collected from tissue culture Lab NIGAB, NARC and field experiment was conducted at two different locations of Gilgit i.e., Babusar and Naltar during the cropping season of 2020-21.

2.2 Laboratory Experiment

In-vitro plants were prepared through tissue culture method by using ex-plant.

2.2.1 MS Media preparation

The potato media was prepared through Murashige and Skoog medium 4.43 supplemented with 30g of sugar and Gibrelic Acid (GA3) 0.5mg per liter. pH adjusted to 5.8. Gelln gum @2g/l was added as a gelling agent and boiled in microwave oven. After cooling the warm medium were poured into test tubes (5x150mm) and flasks (250ml), plugged with cotton. The media was autoclaved at 121^{o} C, 15 PSI for 20 minutes.

2.2.2 Initiation Phase

Cultures were initiated through meristem as the first phase of tissue culture. The explants were sterilized with 20% Clorox and 2-3 drops of tween-20 for 5-8 minutes with continuous shaking. Then washed thoroughly with double distilled water. Sterile forceps and glassware were used, and all process of sterilization completed in laminar air flow.

2.2.3 Multiplication phase

In this phase the invitro explants with one nodal part were re-cultured in the potato medium. After 15-20 days roots developed and processed for hardening and minitubers production.

2.2.4 Hardening phase

Initially in-vitro plants were taken out from nutrient media. First washed thoroughly with tap water and then treated with fungicide (5g/l and then shifted to trays and kept it in green house for two weeks.

2.2.5 Screen House

In this last step of tissue culture these plants were transferred to screen house for tuberization. Among these nucleus tubers six different tuber sizes were selected for field experiment to check the effect of potato nucleus tuber on plant tubers development and seed yield.

2.3 Field Experiment

It was conducted at two different locations/environments during the growing season of 2020-21 at the farms of Agricultural Research Gilgit. The research was carried out to study the influenced of potato tuber size and environment on the plant development and seed yield. Six potato nucleus tuber sizes were sown in the first week of June using RCB Design with three replications at different locations with plant to plant 1 ft and row to row 2.5 ft distance. Each treatment consisted of 90 tubers, 30 tubers in each block and each block contain 3 rows 10 tubers in each. To attain the accurate results uniform cultural practices were used for all treatments for every replication. Plant was watered as per need.

Table 2.1: List of treatments under two different locations.

Treatments	Tuber size
T – 1	0.47g
T - 2	0.84g
T – 3	2.0g
T – 4	5.0g
T – 5	10.0g
T – 6	25.0g

Table 2.2: Randomized Complete Design under two different locations.

Replication 1	Replication 2	Replication 3
T-3	T-1	T-6
T-5	T-6	T-2
T-2	T-4	T-5
T-1	T-3	T-4
T-6	T-2	T-1
T-4	T-5	T-3

2.4 Morphological Parameters.

2.4.1 Days to First Germination

Days to first germination was recorded by counting the days from date of tuber sowing to days to first germination date.

2.4.2 Germination Percentage (%)

Germination percentage was measured by dividing the number of plant emergence by number of tubers sown by multiplying hundred.

2.4.3 Days to Flowering

Days to flowering was calculated from the sowing date till flowering of fifty percent plants in a plot.

2.4.4 Days to Maturity

Days to maturity was calculated from date of sowing to the date of maturity of potato plants.

2.4.5 Leaves per Plant

Leaves per plant were measured by counting the leaves from randomly selected 10 plants from each treatment and mean calculated.

2.4.6 Number of Main Stems

Number of main stems per plant was counted from randomly selected plants from each plot at fifty percent flowering. Those stems which are directly grown from the mother tuber and acted as an independent plant above the soil were considered as main stems.

2.4.7 Plant Height (cm)

Plant height was taken as the mean of 10 plants per plot measured from the surface of the soil to the top-most growing point of plants by means of a meter rod at physiological maturity.

2.4.8 Tubers per Plant

Tubers were counted from randomly selected 10 potato plants from each plot and then their average was used for the final analysis.

2.4.9 Tuber Weight (g)

Single tuber weight was measured for each randomly selected plants tubers on the digital balance and then averaged for data analysis.

2.4.10 Tuber Yield (t/ha)

All tubers from each plot were weighed and converted to tonnes per hectare.

2.4.11 Marketable Tuber Yield (t/ha)

Marketable tuber yield was calculated by weighing all the tubers that were free from disease, defects, cracks, and other physiological disorders and not underweight (100 g) per net plot area and converting into ton per hectare.

2.4.12 Unmarketable Tuber Yield (t/ha)

Unmarketable tuber yield was measured by weighing all tubers other than marketable from each plot and converting into ton per hectare.

2.4.13 Weight of large Tuber (g)

Large tubers were weighted in grams for each randomly selected plant then averaged for data analysis.

2.4.14 Weight of Small Tubers (g)

Small tubers were weighted in grams for each randomly selected plant then averaged for data analysis.

2.5 Physiological Parameters.

2.5.1 Tuber Starch Content (g/100g)

Percentage of tuber starch contents were measured from the specific gravity using the formula: starch percentage = 17.546 + 199.07(specific gravity - 1.0988).

2.5.2 Total Starch Yield (t/h)

Total starch yield was weighted in tons per hectare for each randomly selected plant then averaged for data analysis.

2.5.3 Tuber Dry Matter Content

Tuber dry matter contents were by chopping five tubers into one-to-two-centimeter small cubes and drying two sub samples of 200 g each taken from thoroughly mixed chopped tubers in an oven set at 80^0C for 3 days in two paper bags until a constant weight is reached. Then the percentage of dry matter content for each variety was calculated.

2.5.4 Specific Gravity

Specific gravities were measured by weighing a sample of tuber in air and then re-weighing the sample in water method.

$$\textbf{Specific gravity} = \frac{\text{Weight in Air}}{\text{Weight in Air} - \text{Weight in Water}}$$

2.6 Data analysis

Data analysis was done by using Statistix 8.1 software and two-way ANOVA was applied.

RESULTS

3.1 Morphological Traits

3.1.1 Days to First Germination

Days to first germination was measured by the days from date of tuber sowing to first germination date. Mean value of plantation for days to first germination ranged from 19.66 to 24.00 days in different tuber size treatments. Mean value of treatment 1, 2, 3, 4, 5 and 6 was 24.00, 21.33, 20.66, 20.00, 20.33 and 19.66 days, respectively for Babusar plantation. While mean value of Naltar plantation for days to first germination ranged from 19.33 to 21.00. Mean values of the treatments T1, 2, 3, 4, 5 and 6 were 21.00, 20.67, 19.66, 19.33, 19.66 and 19.66 days, respectively for Naltar plantation (Table 3.1).

Mean value of tuber size and location interaction ranged from 19.33 to 24.00 days for day to first germination. The maximum value for days of first germination was recorded for the T-1 at Babusar plantation (24.00 days), while minimum value of treatment and location interaction was recorded for T-4 at Naltar valley plantation (Table 3.1).

ANOVA showed highly significant ($P<0.01$) difference for the treatments for days to first germination, environment showed significant difference, while treatment and environment interaction showed non-significant variance for days to first germination and coefficient of variation (4.46%) was also recorded for days to first germination as described in (Table 3.2).

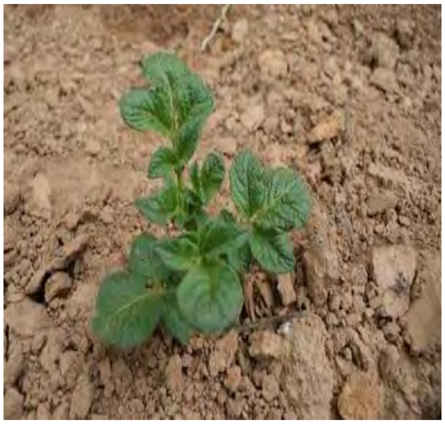

Figure 3.1 First germination

Table 3.1: Mean value for days to first germination of various tuber sizes under two different environmental conditions.

Treatments	No. of days for germination at different locations		Mean
	Babusar	Naltar	
T-1(0.47 g)	24.00[a]	21.00[bc]	**22.50[A]**
T-2(0.84g)	21.33[b]	20.67[bcd]	**21.00[B]**
T-3(2.0g)	20.66[bcd]	20.66[bcd]	**20.66[BC]**
T-4(5.0g)	20.00[bcd]	19.33[d]	**19.67[C]**
T-5(10.0g)	20.33[bcd]	19.66[cd]	**20.00[BC]**
T-6(25.0g)	19.66[cd]	19.66[cd]	**19.67[C]**
Mean	**21.00[A]**	**20.17[B]**	

LSD at 0.05 level for Treatment= 1.0980 LSD at 0.05 level for Locations= 0.6339

LSD at 0.05 level for Treatment and location interaction=1.5528

Table 3.2: Analysis of variance (ANOVA) table for Days to first germination of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	1.5000	0.75000	
Treatment	5	35.2500	7.05000	8.38**
Location	1	6.2500	6.25000	7.43*
Treat ×Environ	5	9.2500	1.85000	2.20[NS]
Error	22	18.5000	0.84091	
Total	35	70.7500		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 4.46%

3.1.2 Germination Percentage (%)

Germination percentage calculated by dividing the number of plant emergence by number of tubers sown multiplied by 100. Mean value of Babusar plantation for germination percentage ranged from 67.33 to 98.33%. Mean value of treatments T- 1, 2, 3, 4, 5 and 6 were

67.33, 83.33, 90.00, 96.67, 98.33 and 98.33%, respectively for Babusar plantation. Mean value of Naltar plantation for germination percentage ranged from 78.33 to 100%, while mean value of treatments T-1, 2, 3, 4, 5 and 6 were 78.33, 96.67, 97.33, 95.00, 99.00 and 100%, respectively for Naltar plantation (Table 3.3).

Mean value of tuber size and location interaction ranged from 67.33 to 100% for germination percentage. The maximum value days of first germination were recorded for T-6 at Naltar plantation (100%), while minimum value of treatment and location interaction was recorded for T-1 on Babusar plantation (Table 3.3).

ANOVA showed highly significant (P<0.01) difference of treatment and significant differences (P<0.05) for location for germination percentage, while treatment and location interaction showed non-significant differences for germination percentage. (Table 3.4). COV was recorded 6.95% for germination (Table 3.4).

Table 3.3: Mean values for germination percentage of various tuber sizes under two different locations.

Treatments	**Germination percentage at different locations**		
	Babusar	**Naltar**	**Mean**
T-1(0.47g)	67.33^{d}	78.33^{c}	$\mathbf{72.83^{C}}$
T-2(0.84g)	83.33^{bc}	96.67^{a}	$\mathbf{90.00^{B}}$
T-3(2.0g)	90.00^{ab}	97.33^{a}	$\mathbf{93.67^{AB}}$
T-4(5.0g)	96.67^{a}	95.00^{a}	$\mathbf{95.83^{AB}}$
T-5(10.0g)	98.33^{a}	99.00^{a}	$\mathbf{98.67^{A}}$
T-6(25.0g)	98.33^{a}	100.00^{a}	$\mathbf{99.17^{A}}$
Mean	$\mathbf{89.00^{B}}$	$\mathbf{94.39^{A}}$	

LSD at 0.05 level for Treatment= 7.63 LSD at 0.05 level for Locations= 4.41

LSD at 0.05 level for Treatment and location interaction=10.80

Table 3.4: Analysis of variance (ANOVA) table for germination percentage of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	144.89	72.444	
Treatment	5	2904.47	580.894	14.29^{**}
Location	1	261.36	261.361	6.43^{*}
Treatment * Location	5	276.47	55.294	1.36^{NS}
Error	22	894.44	40.657	
Total	35	4481.64		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 6.95%

3.1.3 Days to Flowering

Days to flowering was counted when 50% of the plant populations in each plot bloomed. Mean value of Babusar valley plantation for days to flowering ranged from 60.67 to 67.00 days. Mean value of treatments T-1 to 6 was 67.00, 64.34, 63.67, 63.00, 62.34 and 60.67, respectively for Babusar plantations. While mean value of Naltar valley plantation for days to flowering ranged from 61.34 to 68.00 days. Mean value of treatments T-1 to 6 was 68.00, 67.67, 65.67, 65.34, 64.00 and 61.34 days, respectively for Naltar location (Table 3.5).

Mean value of tuber size and location interaction ranged from 60.67 to 68.00 days. Maximum value of days to flowering was recorded in T-1 under Naltar environmental condition (68.00 days). While minimum value of treatment and location interaction was recorded for T- 6 under Babusar environmental condition (Table 3.5).

Analysis of variance showed highly significant differences of treatment and location for days to flowering, while treatment and location interaction showed non-significant (P<0.01) differences for days to flowering. (Table 3.6). Coefficient of variation was recorded 2.02% for days to flowering (Table 3.6).

Figure 3.2 Days to flowering

Table 3.5: Mean value for days to flowering of various tuber sizes under two locations.

Treatments	**Days to flowering at different locations**		**Mean**
	Babusar	**Naltar**	
T-1(0.47g)	67.00[abc]	68.00[a]	**67.500[A]**
T-2(0.84g)	64.34[de]	67.67[ab]	**66.00[AB]**
T-3(2.0g)	63.67[de]	65.67[bcd]	**64.67[BC]**
T-4(5.0g)	63.00[ef]	65.34[cd]	**64.17[C]**
T-5(10.0g)	62.34[efg]	64.00[de]	**63.17[C]**
T-6(25.0g)	60.67[g]	61.34[fg]	**61.00[D]**
Mean	**63.500[B]**	**65.34[A]**	

LSD at 0.05 level for Treatment= 1.56 LSD at 0.05 level for Locations= 0.89

LSD at 0.05 level for Treatment and location interaction=2.20

Table 3.6: Analysis of variance (ANOVA) for days to flowering of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	2.167	1.0833	
Treatment	5	152.250	30.4500	18.02**
Location	1	30.250	30.2500	17.91**
Treat × location	5	6.917	1.3833	0.82NS
Error	22	37.167	1.6894	
Total	35	228.750		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 2.02%

3.1.4 Days to Physiological Maturity

Number of days to physiological maturity was counted when the haulms of 50% of the plant population per plot turned yellowish or showed senescence. Mean value of Babusar plantation for number of days to physiological maturity ranged from 105.33 to 103.34 days. Mean value of treatments T1 to 6 were 105.33, 104.57, 105.34, 104.34, 103.67 and 103.34 days, respectively for Babusar plantation. While mean value of Naltar plantation for days to maturity ranged from 104.00 to 109.33 days. Mean value of treatments T1 to 6 were 109.00, 109.33, 108.34, 106.00, 104.67 and 104.00 days, respectively for Naltar location (Table 3.7).

Mean value of tuber size and location interaction ranged from 103.34 to 109.33 days for days to physiological maturity. Maximum value of days to maturity was recorded for T-2 (0.84 g) under Naltar plantation (109.33), while minimum value of treatment and location interaction was recorded for T-6 (25 g) under Babusar plantation (Table 3.7).

Analysis of variance showed highly significant (P<0.01) differences of treatment and environment individually for days to maturity, while treatment and location interaction showed non-significant (P<0.01) differences for days to maturity. (Table 3.8). Coefficient of variation was recorded 1.23% for days to maturity (Table 3.8)

Table 3.7: Mean values for days to maturity of various tuber sizes under two different locations.

Treatments	**Days to physiological maturity at different locations**		**Mean**
	Babusar	**Naltar**	
T-1(0.47g)	105.33^{bc}	109.00^{a}	$\mathbf{107.17^{A}}$
T-2(0.84g)	104.57^{bc}	109.33^{a}	$\mathbf{107.00^{A}}$
T-3(2.0g)	105.34^{bc}	108.34^{a}	$\mathbf{106.83^{A}}$
T-4(5.0g)	104.34^{bc}	106.00^{b}	$\mathbf{105.17^{B}}$
T-5(10.0g)	103.67^{c}	104.67^{bc}	$\mathbf{104.17^{B}}$
T-6(25.0g)	103.34^{c}	104.00^{bc}	$\mathbf{103.67^{B}}$
Mean	$\mathbf{104.44^{B}}$	$\mathbf{106.89^{A}}$	

LSD at 0.05 level for Treatment= 1.56 LSD at 0.05 level for Locations= 0.90

LSD at 0.05 level for Treatment and location interaction=2.21

Table 3.8: Analysis of variance (ANOVA) for days to physiological maturity of various tuber sizes under two different environmental conditions.

SOV	DF	SS	MS	F value
Replication	2	0.667	0.333	
Treatment	5	71.333	14.2667	8.41^{**}
Location	1	53.778	53.778	31.69^{**}
Treat × Location	5	18.889	3.778	2.23^{NS}
Error	22	37.333	1.6970	
Total	35	182.000		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 1.23%

3.1.5 Number of Leaves per Plant

Number of leaves per plant was determined by counting the number from randomly selected 10 plants from each plot before the start of tuber formation and mean calculated. Mean value of Babusar plantation for number of leaves per plant ranged from 536.67 to 918.40 leaves. Mean value of treatments 1 to 6 were 536.67, 597.20, 637.27, 752.17, 826.43 and 918.40 leaves, respectively for Babusar plantation. While mean value of Naltar plantation for number of leaves ranged from 621.50 to 927.47 leaves. Mean value of treatments 1 to 6 were 621.50, 747.03, 721.73, 772.07, 868.10 and 927.47 leaves, respectively for Naltar location (Table 3.9).

Mean value of treatments (tuber size) and location ranged from 536.67 to 927.47 leaves. Maximum number of leaves were recorded for T-6 (25.0g) under Naltar plantation (927.47), while minimum value of treatment and location interaction were recorded for T-1 (0.47 g) under Babusar plantation (Table 3.9).

Analysis of variance showed highly significant ($P<0.01$) differences of treatments and location individually for number of leaves, while treatment and location interaction showed non-significant ($P<0.01$) differences for number of leaves. (Table 3.10). Coefficient of variation was recorded 6.41% for number of leaves (Table 3.10).

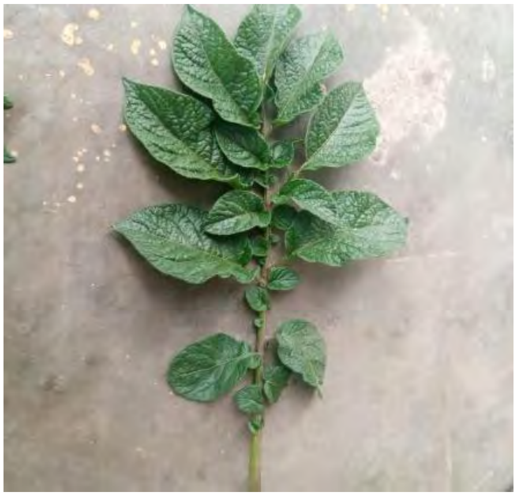

Figure 3.3 Number of leaves

Table 3.9: Mean values for number of leaves of various tuber sizes under two different location trails.

Treatments	No. of leaves per plant at different locations		Mean
	Babusar	Naltar	
T-1(0.47g)	536.67^{f}	621.50^{ef}	**579.08^{e}**
T-2(0.84g)	597.20^{ef}	747.03^{e}	**672.12^{d}**
T-3(2.0g)	637.27^{e}	721.73^{d}	**672.50^{d}**
T-4(5.0g)	752.17^{cd}	772.07^{cd}	**762.12^{c}**
T-5(10.0g)	826.43^{bc}	868.10^{ab}	**847.27^{b}**
T-6(25.0g)	918.40^{a}	927.47^{a}	**922.93^{a}**
Mean	**711.36^{b}**	**776.32^{a}**	

LSD at 0.05 level for Treatment= 57.12 LSD at 0.05 level for Locations= 32.98

LSD at 0.05 level for Treatment and location interaction=80.78

Table 3.10: Analysis of variance (ANOVA) for number of leaves of various tuber sizes under two different plantations.

SOV	DF	SS	MS	F value
Replication	2	20617	10308.3	
Treatment	5	477205	95441.0	41.94^{**}
Location	1	37980	37979.5	16.69^{**}
Treat * Environ	5	20514	4102.8	1.80^{NS}
Error	22	50070	2275.9	
Total	35	606385		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 6.41%

3.1.6 Number of Main Stems per Plant

Number of main stems per plant was counted from randomly selected plants from each plot at 50% flowering. Only stems that directly grown from the mother tuber and acted as an independent plant above the soil were considered as main stems. Stems branching from other stems above the soil were not considered as main stems. Mean value of Babusar plantation for number of main stems ranged from 1.80 to 4.34 stem. Mean value of treatments T-1 to 6 were 1.80, 2.67, 2.87, 3.57, 3.77 and 4.34 stem, respectively for Babusar plantation. While mean value of Naltar plantation for number of main stems ranged from 1.57 to 3.90 stem. Mean value of treatments T-1 to 6 were 1.57, 2.20, 250, 3.57, 3.27 and 3.90 stems, respectively for Naltar location (Table 3.11).

Mean value of tuber size and location ranged from 1.57 to 4.34 stems. Maximum number of main stems was recorded for treatment 6 (25.0 g tuber size) under Babusar plantation (4.34), While minimum value of treatment and location interaction was recorded for treatment 1 (tuber size 0.47 g) under Naltar plantation (Table 3.11).

Analysis of variance showed highly significant ($P<0.01$) differences of treatment and location for number of main stems, while treatment and location interaction showed non-significant ($P<0.01$) differences for number of main stem (Table 3.12). Coefficient of variation was recorded 10.45% for number of main stem (Table 3.12).

Figure 3.4 Number of stems

Table 3.11: Mean values for no. of main stem of various tuber sizes under two different locations.

Treatments	No. of main stem at different locations		Mean
	Babusar	Naltar	
T-1(0.47g)	1.80^{gh}	1.57^{h}	**1.48^{d}**
T-2(0.84g)	2.67^{ef}	2.20^{fg}	**2.43^{c}**
T-3(2.0g)	2.87^{de}	2.50^{ef}	**2.68^{c}**
T-4(5.0g)	3.57^{bc}	3.57^{de}	**3.17^{b}**
T-5(10.0g)	3.77^{bc}	3.27^{cd}	**3.51^{b}**
T-6(25.0g)	4.34^{a}	3.90^{ab}	**4.12^{a}**
Mean	**3.17^{a}**	**2.70^{b}**	

LSD at 0.05 level for Treatment= 0.367 LSD at 0.05 level for Locations= 0.216

LSD at 0.05 level for Treatment and location interaction=0.529

Table 3.12: Analysis of variance (ANOVA) for number of main stems of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	0.8450	0.4225	
Treatment	5	22.020	4.404	46.84**
Location	1	1.960	1.960	20.85**
Treat × location	5	0.267	0.0533	0.57^{NS}
Error	22	2.068	0.0940	
Total	35	27.160		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 10.45%

3.1.7 Plant Height (cm)

Plant height was taken as the average of ten plants per plot measured from the soil surface to the top-most growing point of plants by means of a meter rod at physiological maturity. Mean value of Babusar plantation for plant height ranged from 52.10 to 72.46 cm. Mean value of treatments 1, 2, 3, 4, 5 and 6 were 52.10, 54.30, 58.23, 65.67, 68.40 and 72.46 cm, respectively for Babusar plantation. While mean value of Naltar plantation for plant height ranged from 49.00 to 74.77 cm. Mean value of treatment 1, 2, 3, 4, 5 and 6 were 49.00, 56.33, 55.73, 64.13, 63.40 and 74.77 cm, respectively for Naltar location (Table 3.13).

Mean value treatments and location ranged from 49.00 to 74.77 cm. Maximum plant height was recorded for T-6 (25.0g) under Naltar plantation (74.77 cm), While minimum value of treatment and location interaction was recorded for T-1 (0.47 g) under Naltar plantation (Table 3.13).

Analysis of variance showed highly significant ($P<0.01$) differences of treatment, while non-significant differences of location for plant height, while treatment and location interaction also showed non-significant differences for plant height (Table 3.14). Coefficient of variation was recorded 5.29% for plant height (Table 3.14).

Table 3.13: Mean value for plant height of various tuber size under two different locations.

Treatments	Plant height (cm) at different locations		Mean
	Babusar	Naltar	
T-1(0.47g)	52.10^{fg}	49.00^{g}	**50.55^{d}**
T-2(0.84g)	54.30^{cd}	56.33^{efg}	**55.31^{c}**
T-3(2.0g)	58.23^{de}	55.73^{ef}	**56.98^{c}**
T-4(5.0g)	65.67^{c}	64.13ab^{c}	**64.90^{b}**
T-5(10.0g)	68.40^{bc}	63.40^{cd}	**65.90^{b}**
T-6(25.0g)	72.46^{a}	74.77^{ab}	**73.62^{a}**
Mean	**62.24^{a}**	**60.18^{a}**	

LSD at 0.05 level for Treatment= 3.877 LSD at 0.05 level for Locations= 2.24

LSD at 0.05 level for Treatment and location interaction=5.48

Table 3.14: Analysis of variance (ANOVA) for Plant height of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	14.38	7.189	
Treatment	5	2134.62	426.923	40.71**
Location	1	38.44	38.440	3.67^{NS}
Treat × Location	5	40.51	8.103	0.77^{NS}
Error	22	230.71	10.487	
Total	35	2458.66		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 5.29%

3.1.8 Number of Tubers per Plant

Number of tubers was counted from randomly selected 10 potato plants from each plot and then their average was used for the final analysis. Mean value of Babusar plantation for number of tubers per plant ranged from 5.53 to 11.80 tubers. Mean value of treatments T-1 to 6 were 5.53, 5.53, 8.20, 9.93, 7.00 and 11.80 tubers, respectively for Babusar plantation. While mean value of Naltar plantation for number of tubers per plant ranged from 5.46 to 9.13. Mean value of treatments T-1 to 6 were 5.46, 8.20, 8.20, 6.93, 9.13 and 8.13, respectively for Naltar location (Table 3.15).

Mean value of tuber size and location ranged from 5.46 to 11.80 tubers. Maximum number of tubers per plant were recorded for 25.0 g tuber size under Babusar plantation (11.80), While minimum value of treatment and location interaction was recorded for treatment 1 (0.47 g) under Naltar plantation (Table 3.15).

Analysis of variance showed highly significant (P<0.01) differences of treatment, while non-significant differences of location for number of tubers per plant, while treatment and location interaction also showed non-significant differences for number of tubers per plant (Table 3.16). Coefficient of variation was recorded 12.80% for number of tubers per plant (Table 3.16).

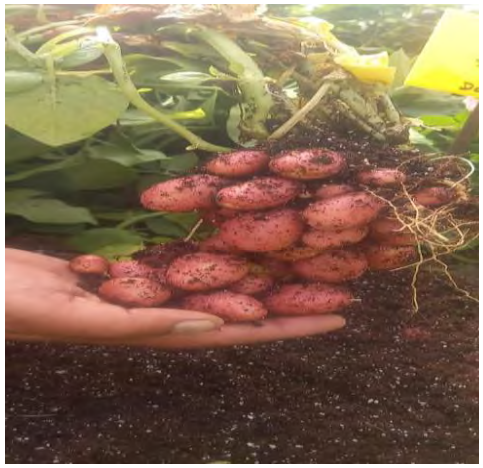

Figure 3.5 Number of tubers

Table 3.15: Mean table for number of tubers per plant of various tuber size under two different environmental conditions.

Treatments	N0. of tuber per plant at different locations		Mean
	Babusar	Naltar	
T-1(0.47g)	5.53^{d}	5.46^{c}	**5.50^{C}**
T-2(0.84g)	5.53^{d}	8.20^{d}	**8.20^{AB}**
T-3(2.0g)	8.20^{bcd}	8.20^{bcd}	**8.00^{A}**
T-4(5.0g)	9.93^{ab}	6.93^{cd}	**8.43^{B}**
T-5(10.0g)	7.00^{bc}	9.13^{abc}	**8.90^{AB}**
T-6(25.0g)	11.80^{a}	8.13^{bcd}	**9.97^{A}**
Mean	**8.72^{A}**	**7.61^{A}**	

LSD at 0.05 level for Treatment= 1.94 LSD at 0.05 level for Locations= 1.12

LSD at 0.05 level for Treatment and location interaction=2.74

Table 3.16: Analysis of variance (ANOVA) for number of tubers per plant of various tuber sizes under two locations.

SOV	DF	SS	MS	F value
Replication	2	0.252	0.1258	
Treatment	5	65.933	13.1867	5.04**
Location	1	11.111	11.111	4.25^{NS}
Treat × Location	5	23.129	23.129	1.77^{NS}
Error	22	57.535	57.535	
Total	35	157.960		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 12.80%

3.1.9 Tuber Weight (g/tuber)

Single tuber weight was measured for each randomly selected plants tubers on the digital balance and then averaged for data analysis. Mean value of Babusar plantation for tuber weight ranged from 60.80 to 84.07 g. Mean value of treatments T-1 to 6 were 63.83, 60.80, 64.00, 65.97, 84.07 and 75.73, respectively for Babusar plantation. While mean value of Naltar plantation for tuber weight ranged from 64.54 to 126.63 g. Mean value of treatments T-1 to 6 were 83.78, 64.15, 84.54, 87.77, 126.63 and 117.73, respectively for Naltar location (Table 3.17).

Mean value of tuber size and location ranged from 60.80 to 126.63 g. Maximum tuber weight was recorded for T-5 (10.0g) under Naltar plantation (126.63g), While minimum value of treatment and location interaction was recorded forT-2 under Babusar plantation (Table 3.17).

Analysis of variance showed non-significant differences of treatments, while significant differences (P<0.05) of location for tuber weight, while treatment and location interaction showed non-significant differences for tuber weight (Table 3.18). Coefficient of variation was recorded 33.91% for tuber weight (Table 3.18).

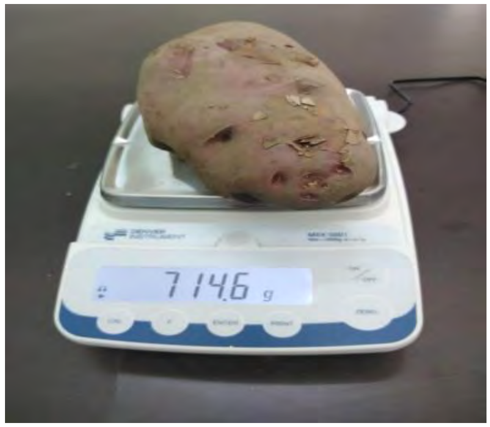

Figure 3.6 Weight of tubers

Table 3.17: Mean values for tuber weight of various tuber sizes under two different locations.

Treatments	Tuber weight (g/tuber) at different locations		Mean
	Babusar	Naltar	
T-1(0.47g)	63.83^{c}	83.78^{abc}	**73.81^{ab}**
T-2(0.84g)	60.80^{c}	64.15^{c}	**62.48^{b}**
T-3(2.0g)	64.00^{c}	84.54^{abc}	**74.27^{ab}**
T-4(5.0g)	65.97^{c}	87.77^{abc}	**76.87^{ab}**
T-5(10.0g)	84.07^{abc}	126.63^{a}	**105.35^{a}**
T-6(25.0g)	75.73^{bc}	117.73^{ab}	**96.73^{a}**
Mean	**69.07^{b}**	**94.10^{a}**	

LSD at 0.05 level for Treatment= 33.12 LSD at 0.05 level for Locations= 19.12

LSD at 0.05 level for Treatment and location interaction=46.84

Table 3.18: Analysis of variance (ANOVA) for tuber weight of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	440.8	220.41	
Treatment	5	7772.9	1554.59	2.03^{NS}
Locations	1	5639.8	5639.76	7.37^{*}
Treat × Location	5	1682.4	336.48	0.44^{NS}
Error	22	16536.5	765.30	
Total	35	32372.5		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 33.91%

3.1.10 Tuber Yield (t/ha)

Mean value of Babusar plantation for tuber yield ranged from 20.10 to 36.46 t/ha. Mean value of treatment T-1, 2, 3, 4, 5 and 6 was 20.10, 24.70, 27.73, 30.66, 33.56 and 36.46, respectively for Babusar plantation. While mean value of Naltar plantation for tuber yield ranged from 23.93 to 37.23 t/ha. Mean value of treatment T-1, 2, 3, 4, 5 and 6 was 23.93, 26.06, 30.20, 24.46, 35.40 and 37.23, respectively for Naltar plantation (Table 3.19).

Mean value of tuber size and location ranged from 20.10 to 37.23 t/ha. Maximum tuber yield was recorded T-6 (25.0 g) under Naltar plantation (37.23 t/ha), While minimum value of treatment and location interaction was recorded for T-1 (0.47 g) under Babusar plantation (Table 3.19).

Analysis of variance showed highly significant (P<0.01) differences of treatment, non-significant differences (p<0.01) of location for tuber yield, while treatment and location interaction also showed non-significant differences for tuber yield (Table 3.20). Coefficient of variation was recorded 15.50% for tuber yield (Table 3.20).

Table 3.19: Mean table for tuber yield of various tuber size under two different locations.

Treatments	Tuber yield at different locations		Mean
	Babusar	Naltar	
T-1(0.47g)	20.10 h	23.93 g	**22.01 f**
T-2(0.84g)	24.70 g	26.06 fg	**25.38 e**
T-3(2.0g)	27.73 ef	30.20 de	**28.96 d**
T-4(5.0g)	30.66 d	24.46 bc	**32.56 c**
T-5(10.0g)	33.56 c	35.40 abc	**34.48 b**
T-6(25.0g)	36.46 ab	37.23 a	**36.85 a**
Mean	**28.87 b**	**31.21 a**	

LSD at 0.05 level for Treatment= 7.29 LSD at 0.05 level for Locations= 4.21

LSD at 0.05 level for Treatment and location interaction=10.32

Table 3.20: Analysis of variance (ANOVA) for tuber yield of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	10.08	5.042	
Treatment	5	958.28	191.656	77.90^{**}
Locations	1	49.47	49.468	20.11^{NS}
Treat × Locations	5	12.09	2.417	0.98^{NS}
Error	22	54.13	2.460	
Total	35	1084.05		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 5.22%

3.1.11 Marketable Tuber Yield (t/ha)

Marketable tuber yield was estimated by the weighing all the tubers free from diseases, insects, defects, and other physiological disorders. It was not underweighting than 100 g per net plot area and converting into tons per hectare. Mean value of Babusar plantation for marketable tuber yield ranged from 17.03 to 34.76 t/ha. Mean value of treatment 1, 2, 3, 4, 5 and 6 was 17.03, 21.96, 24.50, 27.53, 31.46 and 34.76, respectively for Babusar plantation. While mean value of Naltar plantation for marketable tuber yield ranged from 21.53 to 33.63 t/ha. Mean value of treatment 1, 2, 3, 4, 5 and 6 was 21.53, 23.76, 26.83, 31.03, 32.10 and 33.63, respectively for Naltar plantation (Table 3.21).

Mean value of marketable tuber yield and location interaction ranged from 17.03 to 33.63 t/ha. Maximum marketable tuber yield was recorded for T-6 (25.0 g) under Naltar plantation (33.63 t/ha), While minimum value of treatment and location interaction was recorded for T-1 (0.47 g) under Babusar plantation for marketable tuber yield (Table 3.21).

Analysis of variance showed highly significant ($P<0.01$) differences of treatment and location individually for marketable tuber yield, while treatment and location interaction showed significant ($P<0.05$) differences for marketable tuber yield (Table 3.22). Coefficient of variation was recorded 6.73% for marketable tuber yield (Table 3.22).

Table 3.21: Mean values for marketable tuber yield of various tuber sizes under two different locations.

Treatments	Marketable tuber yield at different locations		Mean
	Babusar	Naltar	
T-1(0.47g)	17.03 g	21.53 f	**19.28 f**
T-2(0.84g)	21.96 f	23.76 ef	**22.86 e**
T-3(2.0g)	24.50 def	26.83 de	**25.66 d**
T-4(5.0g)	27.53 d	31.03 c	**29.28 c**
T-5(10.0g)	31.46 bc	32.10 bc	**31.78 b**
T-6(25.0g)	34.76 b	33.63 a	**34.19 a**
Mean	**26.211 b**	**27.66 a**	

LSD at 0.05 level for Treatment= 5.52 LSD at 0.05 level for Locations= 3.18

LSD at 0.05 level for Treatment and location interaction=7.80

Table 3.22: Analysis of variance (ANOVA) for marketable tuber yield of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	5.99	2.994	
Treatment	5	2975.41	595.082	151.77**
Locations	1	369.28	369.280	94.18**
Treat × Location	5	681.26	136.253	34.75**
Error	22	86.26	3.921	
Total	35	64.5212		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 6.73 %

3.1.12 Un-marketable Tuber Yield (t/h)

Unmarketable tuber yield was calculated by weighing all tubers other than marketable from each plot and converting into ton per hectare. Mean value of Babusar plantation for unmarketable tuber yield ranged from 1.70 to 3.40 t/ha. Mean value of treatments T-1 to 6 were 3.40, 2.73, 3.23, 3.13, 2.10 and 1.70, respectively for Babusar plantation. While mean value of Naltar plantation for unmarketable tuber yield ranged from 2.30 to 3.60 t/ha. Mean value of treatments T-1 to 6 were 2.40, 2.30, 3.36, 3.43, 3.30 and 3.60, respectively for Naltar plantation (Table 3.23).

Mean value of treatments and location ranged from 1.70 to 3.60 t/ha. Maximum unmarketable tuber yield was recorded for T-6 (25.0g) under Naltar plantation (3.60 t/ha). While minimum value of treatments and location interaction was recorded for T-6 (25.0 g) under Babusar location for unmarketable tuber yield (Table 3.23).

Analysis of variance showed non-significant differences of treatment and location individually for unmarketable tuber yield, while treatment and location interaction showed non-significant differences for unmarketable tuber yield (Table 3.24). Coefficient of variation was recorded 13.80 % for unmarketable tuber yield (Table 3.24).

Table 3.23: Mean values for unmarketable tuber yield of various tuber sizes under two different locations.

Treatments	Unmarketable tuber yield at different locations		Mean
	Babusar	Naltar	
T-1(0.47g)	3.40 ab	2.40 abc	**2.90 a**
T-2(0.84g)	2.73 abc	2.30 abc	**2.51 a**
T-3(2.0g)	3.23 ab	3.36 ab	**3.30 a**
T-4(5.0g)	3.13 abc	3.43 ab	**3.28 a**
T-5(10.0g)	2.10 bc	3.30 ab	**2.70 a**
T-6(25.0g)	1.70 c	3.60 a	**2.65 a**
Mean	**2.71 a**	**3.06 a**	

LSD at 0.05 level for Treatment= 6.81 LSD at 0.05 level for Locations= 3.93

LSD at 0.05 level for Treatment and location interaction=9.63

Table 3.24: Analysis of variance (ANOVA) for unmarketable tuber yield of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	7.5317	3.76583	
Treatment	5	3.3358	0.66717	0.90^{NS}
Environment	1	1.1025	1.10250	1.48^{NS}
Treat * Environ	5	8.4158	1.68317	2.27^{NS}
Error	22	16.3417	0.74280	
Total	35	36.7275		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV=13.80 %

3.1.13 Weight of Large Tuber (101-550 g)

Large tubers were weighted in grams for each randomly selected plant then averaged for data analysis. Mean value of Babusar plantation for weight of large tuber ranged from 169.00 to

352.43 g. Mean value of treatments T-1 to 6 were 169.00, 253.00, 194.20, 275.57, 296.30, 352.43 g, respectively for Babusar plantation. While mean value of Naltar plantation for weight of large tuber ranged from 257.87 to 487.80 g. Mean value of treatments T-1 to 6 were 257.87, 230.50, 339.70, 360.63, 423.97 and 487.80 g, respectively for Naltar location (Table 3.25).

Mean value of treatments and location ranged from 169.00 to 487.80 g. Maximum weight of large tuber were recorded for T-6 (25.0g) under Naltar plantation (487.80 g), While minimum value of treatments and location interaction were recorded for T-1 (0.47 g) under Babusar plantation (Table 3.25).

Analysis of variance showed significant ($P<0.05$) differences of treatment and location individually for weight of large tuber, while treatment and location interaction showed non-significant differences for weight of large tuber (Table 3.26). Coefficient of variation was recorded 14.28 % for weight of large tuber (Table 3.26).

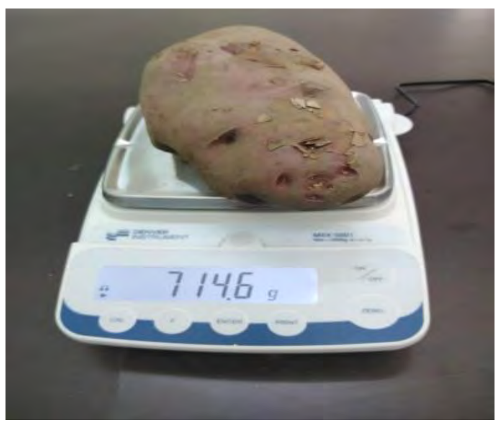

Figure 3.7 Weight of large tuber

Table 3.25: Mean values for weight of large tuber of various tuber sizes under two different locations.

Treatments	Weight of large tuber at different locations		Mean
	Babusar	Naltar	
T-1(0.47g)	169.00^{d}	257.87^{cd}	$\mathbf{213.43^{d}}$
T-2(0.84g)	253.00^{cd}	230.50^{cd}	$\mathbf{241.75^{c}}$
T-3(2.0g)	194.20^{d}	339.70^{bc}	$\mathbf{266.95^{bc}}$
T-4(5.0g)	275.57^{cd}	360.63^{abc}	$\mathbf{318.10^{bc}}$
T-5(10.0g)	296.30^{bcd}	423.97^{ab}	$\mathbf{360.13^{ab}}$
T-6(25.0g)	352.43^{abc}	487.80^{a}	$\mathbf{420.12^{a}}$
Mean	$\mathbf{256.75^{b}}$	$\mathbf{350.08^{a}}$	

LSD at 0.05 level for Treatment= 102.73 LSD at 0.05 level for Locations= 59.60

LSD at 0.05 level for Treatment and location interaction=145.28

Table 3.26: Analysis of variance (ANOVA) for weight of large tuber of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	25155	12577.3	
Treatment	5	115717	23143.5	3.51^{*}
Locations	1	36774	36774.5	5.58^{*}
Treat × location	5	56479	11295.9	0.17^{NS}
Error	22	145053	6593.3	
Total	35	379179		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 14.28%

3.1.14 Weight of Small Tubers (2.0-100 g)

Small tubers were weighted in grams for each randomly selected plant then averaged for data analysis. Mean value of Babusar trail for weight of small tuber ranged from 3.63 to 6.37 g.

Mean value of treatment 1, 2, 3, 4, 5 and 6 was 5.43, 3.63, 6.37, 4.60, 5.86 and 5.70 g, respectively for Babusar plantation. While mean value of Naltar plantation for weight of small tuber ranged from 3.40 to 10.67 g. Mean value of treatment 1, 2, 3, 4, 5 and 6 was 8.50, 3.40, 7.40, 3.73, 6.70 and 10.67 g, respectively for Naltar location (Table 3.27).

Mean value of weight of small tuber and location ranged from 3.40 to 10.67 g. Maximum weight of small tuber was recorded for treatment 6 (25.0 g tuber size) under Naltar plantation (10.67 g), While minimum value of treatment and location interaction was recorded for treatment 2 (tuber size 0.47 g) under Naltar plantation (Table 3.27).

Analysis of variance showed non-significant differences of treatment and location individually for weight of small tuber, while treatment and location interaction also showed non-significant differences for weight of small tuber (Table 3.28). Coefficient of variation was recorded 15.92 for weight of small tuber (Table 3.28).

Figure 3.8 Weight of small tuber

Table 3.27: Mean values for weight of small tubers of various tuber sizes under two different locations.

Treatments	Weight of small tuber at different locations		Mean
	Babusar	Naltar	
T-1(0.47g)	5.43 [a]	8.50 [a]	**6.96[a]**
T-2(0.84g)	3.63 [a]	3.40 [a]	**3.51[a]**
T-3(2.0g)	6.37 [a]	7.40 [a]	**6.88[a]**
T-4(5.0g)	4.60 [a]	3.73 [a]	**4.16[a]**
T-5(10.0g)	5.86[a]	6.70 [a]	**6.28[a]**
T-6(25.0g)	5.70[a]	10.67 [a]	**8.18[a]**
Mean	**5.27[a]**	**6.73[a]**	

LSD at 0.05 level for Treatment= 6.17 LSD at 0.05 level for Locations= 3.56

LSD at 0.05 level for Treatment and location interaction= 8.72

Table 3.28: Analysis of variance (ANOVA) for weight of small tubers of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	17.807	8.9036	
Treatment	5	82.622	16.524	0.67^{NS}
Locations	1	33.640	33.640	0.36^{NS}
Treat × Locations	5	41.607	8.3213	0.34^{NS}
Error	22	544.333	24.7424	
Total	35	720.009		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 15.92%

3.2 Physiological Parameters

3.2.1 Tuber Dry Matter Contents:

Tuber dry matter contents were determined by chopping five tubers into 1-2 cm small cubes and drying two sub samples of 200g each taken from thoroughly mixed chopped tubers in an oven set at 80^0C for 72 hours in two paper bags until a constant weight is reached. Then the percentage of dry matter content for each variety was calculated. Mean value of Babusar plantation for tuber dry matter contents ranged from 21.96 to 24.50 %. Mean value of treatments T-1 to 6 was 23.08, 21.96, 23.05, 24.50, 24.23 and 22.30, respectively for Babusar plantation. While mean value of Naltar plantation for tuber dry matter contents ranged from 21.41 to 24.53 %. Mean value of T-1 to 6 was 21.41, 22.49, 24.53, 21.95, 22.26 and 23.18 %, respectively for Naltar plantation (Table 3.29).

Mean value of treatments and location ranged from 21.41 to 24.53 %. Maximum tuber dry matter content was recorded for T-3 (2.0g) under Naltar plantation (24.53%). While minimum value of treatment and location interaction was recorded for T-1 (0.47g) under Naltar plantation (Table 3.29).

Analysis of variance showed non-significant differences of treatments and location individually for tuber dry matter content, while treatment and location interaction also showed non-significant differences for tuber dry matter content (Table 3.30). Coefficient of variation was recorded 7.06% for tuber dry matter content (Table 3.30).

Table 3.29: Mean value for tuber dry matter contents of various tuber sizes under two different locations.

Treatments	Tuber dry matter content at different locations		Mean
	Babusar	Naltar	
T-1(0.47g)	23.08^{ab}	21.41^{a}	**22.24^{a}**
T-2(0.84g)	21.96^{ab}	22.49^{b}	**22.23^{a}**
T-3(2.0g)	23.05^{ab}	24.53^{ab}	**23.79^{a}**
T-4(5.0g)	24.50^{a}	21.95^{ab}	**23.22^{a}**
T-5(10.0g)	24.23^{a}	22.26^{ab}	**23.24^{a}**
T-6(25.0g)	22.30^{ab}	23.18^{ab}	**22.74^{a}**
Mean	**23.19^{a}**	**22.64^{a}**	

LSD at 0.05 level for Treatment= 1.93 LSD at 0.05 level for Locations= 1.12

LSD at 0.05 level for Treatment and location interaction=2.74

Table 3.30: Analysis of Variance (ANOVA) for tuber dry matter content of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	3.165	1.582	
Treatment	5	11.563	2.312	0.88^{NS}
Locations	1	2.739	2.739	1.05^{NS}
Treat × Locations	5	21.960	4.392	1.68^{NS}
Error	22	57.612	2.619	
Total	35	97.039		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 7.06%

3.2.2 Specific Gravity

Specific gravities were measured by weighing a sample of tuber in air and then re-weighing the sample in water method. Mean value of Babusar plantation for specific gravity ranged from 1.06 to 1.09. Mean value of treatments 1, 2, 3, 4, 5 and 6 were 1.06, 1.09, 1.07, 1.07, 1.09 and 1.09, respectively for Babusar plantation. While mean value of Naltar plantation for specific gravity ranged from 1.08 to 1.10. Mean value of treatments 1, 2, 3, 4, 5 and 6 were 1.09, 1.08, 1.08, 1.10, 1.09 and 1.09, respectively for Naltar plantation (Table 3.31).

Mean value of tuber size and location interaction ranged from 1.06 to 1.10. Maximum specific gravity was recorded for treatment 4 (5.0 g tuber size) under Naltar plantation (1.10), While minimum value of treatment and location interaction was recorded for treatment 1 (tuber size 0.47 g) under Babusar plantation (Table 3.31).

Analysis of variance showed non-significant differences of treatments and location for specific gravity, while treatments and location interaction also showed non-significant differences for specific gravity (Table 3.32). Coefficient of variation was recorded 1.37% for specific gravity (Table 3.32).

Table 3.31: Mean value for specific gravity of various tuber sizes under two different locations.

Treatment/Location	**Specific gravity at different locations**		**Mean**
	Babusar	**Naltar**	
T-1(0.47g)	1.06^{b}	1.09^{a}	**1.076^{b}**
T-2(0.84g)	1.09^{a}	1.08^{ab}	**1.09^{a}**
T-3(2.0g)	1.07^{ab}	1.08^{ab}	**1.08^{a}**
T-4(5.0g)	1.07^{ab}	1.10^{a}	**1.09^{a}**
T-5(10.0g)	1.09^{a}	1.09^{a}	**1.09^{a}**
T-6(25.0g)	1.09^{ab}	1.09^{a}	**1.09^{a}**
Mean	**1.08^{a}**	**1.09^{a}**	

LSD at 0.05 level for Treatment= 0.018 LSD at 0.05 level for Locations= 0.01

LSD at 0.05 level for Treatment and location interaction=0.03

Table 3.32: Analysis of variance (ANOVA) for specific gravity of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	0.00079	0.0000395	
Treatment	5	0.00119	0.0000238	1.07^{NS}
Location	1	0.00066	0.000065	2.96^{NS}
Treat × Location	5	0.0014	0.0000298	1.34^{NS}
Error	22	0.00489	0.0000222	
Total	35	0.00903		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 1.37%

3.2.3 Tuber Starch Content (g/100g)

The percentage of starch contents were calculated from the specific gravity using the formula: starch percentage = 17.546 + 199.07(specific gravity - 1.0988). Mean value of Babusar plantation for tuber starch content ranged from 12.93 to 16.53 g/100g. Mean value of treatments T-1 to 6were 13.07, 16.13, 12.93, 13.20, 16.53 and 14.60 g/100g, respectively for Babusar plantation. While mean value of Naltar plantation for tuber starch content ranged from 14.40 to 16.80 g/100g. Mean value of treatments T-1 to 6 were 16.00, 14.50, 14.40, 16.80, 16.49 and 15.33 g/100g, respectively for Naltar plantation (Table 3.33).

Mean value of treatments (tuber starch content) and location ranged from 12.93 to 16.80 g/100g. Maximum tuber starch content was recorded for T-4 (5.0g) under Naltar plantation (16.80 g/100g). While minimum value of treatments and location interaction were recorded for T-3 (0.47g) under Babusar plantation (Table 3.33).

Analysis of variance showed significant differences of treatments and location for tuber starch content, while treatments and location interaction also showed non-significant differences for tuber starch content (Table 3.34). Coefficient of variation was recorded 19.09% for tuber starch content (Table 3.34).

Table 3.33: Mean values for tuber starch content of various tuber sizes under two different locations.

Treatments	Tuber starch content g/100g at different locations		Mean
	Babusar	Naltar	
T-1(0.47g)	13.07[a]	16.00[a]	**14.53[a]**
T-2(0.84g)	16.13[a]	14.50[a]	**15.35[a]**
T-3(2.0g)	12.93[a]	14.40[a]	**13.67[a]**
T-4(5.0g)	13.20[a]	16.80[a]	**15.00[a]**
T-5(10.0g)	16.53[a]	16.49[a]	**16.51[a]**
T-6(25.0g)	14.60[a]	15.33[a]	**14.97[a]**
Mean	**14.41[a]**	**15.60[a]**	

LSD at 0.05 level for Treatment= 3.43 LSD at 0.05 level for Locations= 1.98

LSD at 0.05 level for Treatment and location interaction=4.85

Table 3.34: Analysis of variance (ANOVA) for tuber starch content of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	3.1145	1.5572	
Treatment	5	30.4201	6.08403	6.06**
Locations	1	5.0027	5.0027	4.98*
Treat × Locations	5	3.8816	0.77632	0.77^{NS}
Error	22	22.1023	1.0047	
Total	35	64.5212		

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 19.09%

3.2.4 Total Starch Yield (t/h)

Total starch yield was weighted in tons per hectare for each randomly selected plant then averaged for data analysis. Mean value of Babusar trial for total starch yield ranged from 2.45 to

5.52. Mean value of treatments T-1 to 6 were 2.45, 3.96, 3.57, 4.03, 5.52 and 5.32, respectively for Babusar plantation. While mean value of Naltar plantation for total starch yield ranged from 3.86 to 5.85. Mean value of treatments T-1 to 6 were 3.86, 3.81, 4.34, 5.78, 5.85 and 5.68, respectively for Naltar location (Table 3.35).

Mean value of treatments (total starch yield) and location ranged from 2.45 to 5.85 t/h. Maximum total starch yield was recorded for T-5 (10.0g) under Naltar plantation (5.85 t/h), while minimum value of treatments and location interaction was recorded for T-1 (0.47 g) under Babusar plantation (Table 3.35).

Analysis of variance showed highly significant differences of treatments and significant difference of location for total starch yield, while treatments and location interaction also showed non-significant differences for total starch yield (Table 3.36). Coefficient of variation was recorded 12.19 % for total starch yield (Table 3.36).

Table 3.35: Mean values for total starch yield (t/h) of various tuber sizes under two different locations.

Treatments	Total starch yield (t/h) at different locations		Mean
	Babusar	Naltar	
T-1(0.47g)	2.45 f	3.86 cdef	**19.28 f**
T-2(0.84g)	3.96 cdef	3.81def	**22.86 e**
T-3(2.0g)	3.57 ef	4.34abcde	**25.66 d**
T-4(5.0g)	4.03bcdef	5.78a	**29.28 c**
T-5(10.0g)	5.52 abc	5.85a	**31.78 b**
T-6(25.0g)	5.32abcd	5.68ab	**47.60 a**
Mean	**4.1439 b**	**4.8894 a**	

LSD at 0.05 level for Treatment= 2.19 LSD at 0.05 level for Locations= 1.27

LSD at 0.05 level for Treatment and location interaction=3.11

Table 3.36: Analysis of variance (ANOVA) for total starch yield (t/h) of various tuber sizes under two different locations.

SOV	DF	SS	MS	F value
Replication	2	3.1145	1.55726	
Treatment	5	30.4201	6.08403	6.06**
Locations	1	5.0027	5.00268	4.98**
Treat × Locations	5	3.8816	0.77632	0.77 NS
Error	22	22.1023	1.00465	
Total	35			

**= Highly significant (<0.01) *=significant (<0.05) NS= non-significant

CV= 12.1

DISCUSSION

To characterize the germplasm for quantitative and qualitative characteristics, there are many parameters; morphologically, physiologically as well as molecular level (Ciancolini*et al.,* 2012). Various tuber sizes have been investigated for morphological traits including; days to first germination (Ahmadizadeh and Felenji, 2011), germination percentage (Khan *et al.,* 2010), number of main stem (Gulluoglu and Arioglu, 2009), plant height (Masarirambi et al. 2012 and Zebenay, 2015), days to physiological maturity (Masarirambi et al. 2012), number of tubers per plant (Michael et al, 2011 and Zebenay, 2015), days to flowering (Plantenga, 2019), number of leaves per plant (Masarirambi *et al.,* 2012), weight of large tuber (Nasir and Akassa, 2018), weight of small tuber (Ebrahim, 2018), unmarketable tuber yield (Ebrahim *et al.,* 2018) , marketable tuber yield (Nasir and Akassa, 2008) and total yield ton per hectare (Michael *et al.,* 2012 and Ebrahim et al. 2018). Physiological traits including starch content (Regierer *et al.,* 2002), total starch yield (Gedif et al, 2014), tuber dry matter content (Mostofa *et al.,* 2019; Ebrahim *et al.,* 2018) and specific gravity (Mostofa *et al.,* 2019). These methods in solanum species especially at large scales required minimum manpower and cheap method for routine monitoring. In-vitro multiplication and effect of tuber size on potato seed production.

4.1 Morphological Parameters

The analysis of variance (ANOVA) of the potato yield traits and its components showed highly significant differences among treatments, significant difference for location and non-significant difference for interaction between treatment and location. Some parameters showed better performances in Naltar plantation such as germination percentage, number of leaves per plant, tuber weight (g/tuber), tuber yield (t/h), marketable tuber (t/h) and weight of large tuber (g), while the other morphological parameters like as, days to first emergence, number of main stem per plant, plant height, number of tubers per plant, unmarketable tuber (t/h) and weight of small tuber (g) were better in Babusar plantation.

In some cases, our findings congruent with previous studies that have successfully used morphological traits such as tuber size and tuber weight to acquire the effect of potato nucleus tuber size on plant development and seed yield (Mu *et al.,* 2018).

Potato tuber size influenced by the number of days to first germination. So, large potato tubers taken minimum number of days to germinated, while small seed potato tubers have taken maximum number of days to germination. Current results align with the studies conducted by (Masarirambi *et al.,* 2012), these studies showed the number of days to seed germination is influenced by the environmental factors such as, tuber size, climatic conditions, and geographic locations. As tuber size increases, there is a marked decrease in days to germination. This effect is due to more carbon source is available to germinate the potato plant. It is, therefore, essential to understand how tuber size effect the germination days. Machado *et al.,* (2007) also found significant difference of seed tuber size for days to first germination or days to emergence. Meanwhile the size of seed tubers influenced crop establishment, it followed those plants from larger seed tubers had less days to first emergence compared to smaller seed.

Large and extra-large seed showed maximum germination, while small to medium sized seed gave less percentage of germination. Obtained result correlate with (Khan *et al.,* 2010), the germination percentage of tubers is more in the case of large tubers. This was because germination percentage was largely dependent on the utilization of reserve material and metabolites in the mother tuber (Kabir *et al.,* 2004). Larger seed tubers have a greater initial meristematic potential and a greater amount of reserve material than smaller seed tubers (Aighewi *et al.,* 2015). Present results higher relative growth rates showed by large seed than small seed in the pre-emergence as well as the post-emergence durations, because large seed is associated with large embryo axis, leaf primordial and cotyledon area. Large and extra-large seed tubers had slightly longer and thicker sprouts at planting time and this help in earlier germination and crop establishment. Masarirambi *et al.,* (2012) also found similar results that relatively large tuber shows their earlier germination.

Pacini and Dolferus, 2016 described that as size of tuber is increasing, the plant took less days to flowering and more days in decreasing size. This is because of the tubers contain enough nutrients and enzymes for completing the vegetative growth and reach to reproductive phase. Tekalign and Hammes, (2005) also reported that earliness in flowering is controlled by factors including genetic and environmental factors. Regarding seed tuber size, large tuber sizes significantly required a smaller number of days to reach flowering stage whereas, medium to small seed tuber sizes significantly required a greater number of days to reach flowering stage. Ebrahim *et al.,* (2018) reported that a greater number of days to flowering was reported for small tuber size.

Tuber size significantly affect the days to physiological maturity. With increasing the size of tuber, the days to maturity decreases. Masarirambi *et al.,* (2012) reported that large and extra-large seed tubers had slightly longer and thicker sprouts at planting time and this help in earlier maturity and crop growth. Tekalign and hammes, (2005) reported that the use of large seed tuber size required short time to reach maturity while smaller seed tuber size required long duration to mature physiologically. While Duffy and Cassells (2000) found medium size tubers took maximum days to maturity.

Masarirambi *et al.,* (2012) reported that the size of seed tubers influenced crop growth, it followed those plants from larger seed tubers had a greater number of leaves compared to smaller seed. Large size tubers contain a greater number of buds result large number of stems hence large number of branches and leaves while, a smaller number of leaves in small size tubers. Number of leaf production was highest in large seed tubers, decreasing almost uniformly as we moved from medium to small seed tubers. This phenomenon was also endorsed to the efficient allocation of more biomass in larger than in small seed tubers.

Gulluoglu and Arioglu, 2009 explained that the phenomenon of varying number of stems based on different size of tubers. Large size tubers contain a greater number of buds as a resulted large number of mother stems, while small size tubers contain a smaller number of mother stem as like our findings. Larger seed tubers were found to produce a greater number of sprouts than smaller seed tubers hence, compatibly a greater number of stems per plant. Takalign and Hammes, (2005) reported that Seed tuber size significantly affected the main stem number such that the more stem numbers were recorded from larger tuber size, while the smaller number of stems were recorded from smaller tuber size. It would be positive correlation between the tuber size and number of main stems.

Masarirambi *et al.,* (2012) reported that plants from large seed size significantly tall plants than plants derived from smaller seed size. There was a steady increase in the plant height as it moved from small tuber to large tubers. Zebenay, (2015) concluded in his study regarding tuber size, that the maximum plant height of a potato plants was obtained from large seed tuber sizes whereas shorter plants height was obtained from small seed tuber sizes. Large seed tuber sizes produced the tallest plants which were higher than medium and small seed tuber sizes by about 4.9% and 8.9%, respectively. The variation in plant height might be due to the higher food reserves in tubers with larger size than small and medium seed tuber sizes which enhanced vegetative growth of the plant including the height of the plant.

Plants from large and very large seed tubers were significantly different in terms of number of tubers per plant. This phenomenon was attributed to more vigorous and rapid growth of larger than smaller seed potato tubers. Michael *et al.,* (2011) and Zebenay, (2015) reported that plants grown from large seed tuber size produced high total tuber number per plant whereas small seed tuber size produced low total tuber numbers per plant. Large seed tuber size significantly exceeded in producing total number of tubers. This might be due to the maximum nutrients availability in the case of large tuber seed.

Masarirambi *et al.,* (2012) reported in their results that seed tuber size significantly influence on potato weight. Plants from large seed size significantly increase the weight of potato and decrease in small seed size. Hence yield performance was greatest by larger tuber size. Tuber weight was affected by seed tuber size compared to medium and small tuber seed (Michael *et al.,* 2012). Ebrahim *et al.,* (2018) reported that seed tuber size and varieties had a very highly significant influence on total tubers yield in tons per hectare. Plants established from large seed tuber produced smaller but numerous tubers whereas those established from small to medium sized seed produced few but large tubers, reported by (Khan *et al.*, 2010).

Nasir and Akassa, (2008) reported that larger tuber seed increased the high marketable tuber quantity compared to smaller tubers and this increased their photosynthesis efficiency for higher photo assimilation production and ultimately resulted in increased more marketable tuber yield. Researchers graded tubers into four categories according to diameter: category C1 for those below 40 mm; C2 for those between 40 and 50 mm; C3 for 50–60 mm and C4 for those above 60 mm. The tuber size categories C2 and C3 were considered marketable tubers.

Ebrahim *et al.,* (2018) reported that seed tuber size significantly affected unmarketable tuber yield. The maximum unmarketable tuber yields were recorded from smaller seed tuber size, while the minimum unmarketable tuber yields were recorded from larger seed tuber size and their results are parallel to our results.

Nasir and Akassa, (2018) reported in their conclusion that weight of large tuber and weight of small tuber was affected by seed size of tubers. Weight of large tubers has been increased with increasing the tuber seed size. Similarly, with small tuber seed size decrease the weight of large tuber. This might be due to the ability and availability of nutrients from mother tubers to produce good quality large tubers. Researchers reported that smaller tubers were less likely to produce good quality small tubers. He concluded that small size tuber seed reduce the weight of both small tuber and large tuber. This might be due to many factors in which nutrient availability is one of the most important factors.

4.2 Physiological Parameter

The analysis of variance (ANOVA) of the potato tuber starch content, specific gravity and tuber dry matter content showed non-significant differences among treatments and location while treatment and location interaction also non-significant difference. Total starch yield showed highly significant difference for treatment and significant difference for location. Mainly physiological parameters showed almost equal performances in both Naltar and Babusar plantation.

Mostofa *et al.,* (2019) found significant variation among different tuber sizes on tuber dry matter content. Maximum dry matter content was obtained by large tuber size and minimum dry matter was obtained by smaller tuber seed size. Ebrahim *et al.,* (2018) reported that seed tuber size had a highly significant effect on tuber dry matter content. Large seed tuber size produced the highest tuber dry matter content.

Mostofa *et al.,* (2019) reported significant differences among different tuber sizes on the specific gravity of tuber. The highest specific gravity (1.10 g cm^3) of tuber was exhibited by >5g and lowest (1.06 g cm3) was exhibited by 0.47g. The relationship between specific gravity and tuber is not clear from the literature. Sawyer and Collin (1960) stated that potato tuber specific gravity appeared to be related to tuber size; however, they found relationship between varietal response and varietal specific gravity.

Regierer *et al.,* (2002) found statistically variation among various seed tuber sizes for tuber starch content which contrast with my results. Cottrell *et al.,* (1995) concluded on their experiments that tuber starch contents are significantly affected by genotypic variation, number of growing conditions in which the tuber size is main factor that effect the tuber starch content.

Tuber size is important to total starch yield (Hamunyela *et al.,* 2020). From their experiment, size increased with tuber size. Furthermore, an interaction between cultivar and tuber size was observed, implying that the magnitude of starch yield due to tuber size depends on the tuber seed size.

SUMMARY

Potato (*Solanum tuberosum*), most dominant tuber crop in the world, are highly contributed crop play a vital role in maintaining human nutrition and food security. The specific objectives of this study were to: In-vitro multiplication of different potato varieties and optimization of tuber size for multiplication in field under different environmental conditions.

The experimental material, mini tubers were collected from tissue culture Lab NIGAB, NARC and field experiment was conducted at two different locations of Gilgit i.e Babusar and Naltar during the cropping season of 2020-21. In-vitro plants were prepared through tissue culture method by using ex-plant. Initially in-vitro plants were taken out from nutrient media. First washed thoroughly with tap water and then treated with fungicide (5g/l and then shifted to trays and kept it in green house for 2 weeks. In this last step of tissue culture these plants were transferred to screen house for tuberization. Among these nucleus tubers six different tuber sizes were selected for field experiment to check the effect of potato nucleus tuber on plant tubers development and seed yield.

Field experiments were carried out at two different locations during the growing season of 2020-21 at the farms of Agricultural Research Gilgit. The experiment was carried out to study the in-vitro multiplication and effect of tuber size on potato seed production. Six potato nucleus tuber sizes were sown in the first week of June using Randomized Complete Block Design (RCBD) with three replications at different locations with plant to plant1 ft. and row to row 2.5 ft. distance. Each treatment consisted of 90 tubers, 30 tubers in each block and each block contain 3 rows 10 tubers in each. To achieve the maximum accurate results uniform agronomic practices were performed for all treatments in each replication. Crop was irrigated as per requirements. Data was recorded from 10 randomly selected plants for each treatment in each replication.

Morphological parameters consist of days to first germination, germination percentage (%), days to flowering, days to physiological maturity, number of leaves, number of main stems, plant height (cm), number of tubers per plant, tuber weight (g), tuber yield (t/ha), marketable tuber yield (t/ha), unmarketable tuber yield (t/ha), weight of large tuber (g), weight of small

tubers (g). Physiological parameters were consisting of tuber starch content (g/100g), total starch yield (t/h), tuber Dry matter content, and specific gravity.

Data of the studied traits were analyzed by using statistical package, Statistix 8.1, to calculate the genetic variability for different parameters of potato treatments. Least significant differences (LSD) test was also applied.

Analysis of variance showed significant difference for the treatments for days to first germination, germination percentage, days to flowering, days to physiological maturity, number of leaves per plant, number of main stems per plant, plant height, number of tubers per plant, tuber yield, marketable tuber yield, size of large tubers, tuber starch content, total starch yield,

Environment showed significant difference for days to first germination, germination percentage, days to flowering, days to physiological maturity, number of leaves per plant, number of main stems per plant, tuber weight, marketable tuber yield, size of large tuber, tuber starch content, total starch yield, while treatment and environment interaction showed non-significant differences for marketable tuber yield.

ANOVA showed non-significant differences for treatment and location interaction of days to first germination, treatment and location interaction of germination percentage, treatment and location interaction of days to flowering, treatment and location interaction of days to physiological maturity, treatment and location interaction of number of leaves per plant, treatment and location interaction of number of main stem per plant, location of plant height, treatment and location interaction of plant height, location of number of tubers per plant, treatment and location interaction of number of tubers per plant, treatment of tuber weight, treatment and location interaction of tuber weight, location of tuber yield, treatment and location interaction of tuber yield, treatment, location and interaction of unmarketable tuber yield, treatment location interaction of size of large tuber, treatment, location and interaction of size of small tuber, treatment, location and interaction of tuber dry matter content, treatment, location and interaction of specific gravity, interaction of tuber starch content, interaction of total starch yield. Present results showed that the size of seed tubers influenced crop establishment, it followed those plants from larger seed tubers had good performance compared to smaller seed.

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