T.R.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL GENETIC ENGINEERING

SELECTION OF POTATO BREEDING LINES HAVING HIGH TUBER YIELD, TUBER QUALITY, AND RESISTANT TO PVY AND PVX

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MASTER THESIS

NİĞDE ÖMER HALISDEMIR UNIVERSITY
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Master Thesis

Supervisor
Prof. Dr. Mehmet Emin ÇALIŞKAN

August 2021

The study titled “Selection of Potato Breeding Lines Having High Tuber Yield, Tuber Quality, and Resistant to PVY and PVX” and presented by Yahye Abdullahi ISSE under the supervision of Prof. Dr. Mehmet Emin ÇALIŞKAN has been accepted as a Master of Science thesis by the jury at the Department of Agricultural Genetic Engineering of Niğde Ömer Halisdemir University, Graduate School of Natural and Applied Sciences.

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This thesis has been found appropriate at the date of 23/08/2021 by the jury mentioned above who have been designated by the Board of Directors of Graduate School of Natural and Applied Sciences and has been confirmed with the resolution of the Board of Directors dated …./…./2021 and numbered ……………

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DECLARATION OF THESIS

I declare that this thesis was written by me, and I further confirm that all information included in this Thesis is scientific and is in accordance with the University's rules and regulations. Any information and materials used from external sources as well as help received and all sources used in preparing and finalizing this thesis research work have been duly acknowledged in the thesis.

Yahye Abdullahi ISSE

SUMMARY

SELECTION OF POTATO BREEDING LINES HAVING HIGH TUBER YIELD, TUBER QUALITY, AND RESISTANT TO PVY AND PVX

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Graduate School of Natural and Applied Sciences
Department of Agricultural Genetic Engineering

Supervisor: Prof. Dr. Mehmet Emin ÇALIŞKAN

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This study was conducted in 2020 to determine yield and quality traits, and resistance to two important viruses (PVY and PVX). of 28 potato breeding lines and 5 standard cultivars in Nigde conditions. All breeding lines and cultivars were screened for resistance to two viruses using three SSR markers (1Rx1, 106Rx2, and STM0003). There were highly significant ($p \leq 0.01$) effects of genotype on yield and quality traits of potato genotypes tested. The highest total (48.23 t/ha) and marketable tuber yields (46.52 t/ha) were obtained from the breeding line MEÇ17011.11, while the lowest total (15.15 t/ha) and marketable yields (13.01 t/ha) were obtained from the breeding line MEÇ17023.01. Molecular marker analysis showed that all breeding lines and cultivars have resistance genes for PVX, while PVY has resistance to 11 out of 33 genotypes. In addition, MEÇ17011.11, MEÇ17011.13, MEÇ17023.01, MEÇ17023.04, MEÇ17065.02, MEÇ17075.01, MEÇ17075.08, MEÇ17076.02, MEÇ17076.03, MEÇ17078.03, MEÇ17080.01 genotypes have resistance genes for both PVY and PVX. The breeding lines MEÇ17011.11 and MEÇ17078.03 and were found as promising cultivar candidates due to their high tuber yield and resistance genes for both viruses (PVY, PVX).

Keywords: Potato, breeding line, adaptation, selection, PVX, PVY

ÖZET

YÜKSEK YUMRU VERİMİ VE KALİTESİNE SAHİP PVY VE PVX DAYANIKLI PATATES HATLARININ SELEKSİYONU

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Bu çalışma, 28 patates ıslah hattı ve 5 çeşidin verim ve kalite özellikleri ile iki önemli virüs (PVY ve PVX) hastalığına dayanıklılığını belirlemek amacıyla 2020 yılında Niğde koşullarında yürütülmüştür. Tüm ıslah hatları ve çeşitler iki virüs hastalığına dayanıklılık yönünden üç SSR markörü (1Rx1, 106Rx2, and STM0003) ile taranmışlardır. Denemeye alınan patates genotiplerinde verim ve kalite özellikleri açısından önemli ($p \leq 0.01$) farklılıklar olduğu belirlenmiştir. En yüksek toplam (48.23 t/ha) ve pazarlanabilir (46.52 t/ha) yumru verimi MEÇ17011.11 kodlu ıslah hattından elde edilirken, en düşük toplam (15.15 t/ha) ve pazarlanabilir (13.01 t/ha) yumru verimi ise MEÇ17023.01 kodlu ıslah hattından elde edilmiştir. Moleküler markör analiz sonuçları tüm ıslah hatları ve çeşitlerin PVX dayanıklılık genlerine sahip olduğunu ancak 33 genotipin 11 tanesinde PVY dayanıklılık geninin bulunduğunu göstermiştir. Ayrıca MEÇ17011.11, MEÇ17011.13, MEÇ17023.01, MEÇ17023.04, MEÇ17065.02, MEÇ17075.01, MEÇ17075.08, MEÇ17076.02, MEÇ17076.03, MEÇ17078.03, MEÇ17080.01 ıslah hatlarının hem PVY hem de PVX dayanıklılık genlerine sahip olduğu belirlenmiştir. Sonuç olarak MEÇ 17011.11 ve MEÇ17078.03 kodlu ıslah hatlarının hem yüksek yumru verimi hem de her iki virüse karşı dayanıklılık genlerine sahip olması nedeniyle tescil başvurusu yapılabilecek ümitvar çeşitler olduğu belirlenmiştir.

Anahtar Kelimeler: Patates, ıslah hatları, adaptasyon, seleksiyon, PVX, PVY

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SYMBOL AND ABBREVIATION

Symbols	**Description**
°C	Degree centigrade
Mg/ml	Milligram/ millilitre
mM	Milli molar
%	Percentage

Abbreviation	**Description**
CV	Coefficient of Variation
DF	Degree of Freedom
DMC	Dry Matter Concentration
DNA	Deoxyribonucleic Acid
FAO	Food and Agricultural Organization
FCT	First Class Tuber
HP	Hypersensitivity Genes
LSD	Least Significant Difference
MTN	Medium Tuber Number
MTW	Mean Tuber Wight
MTY	Marketable Tuber Yield
NSP	Number of Stem per Plant
NTP	Number of Tuber per Plant
PCR	Polymerase Chain Reaction
pH	Power of hydrogen proton
PVX	Potato Virus X
PVY	Potato Virus Y
SCT	Second Class Tuber
SE	Stand Establishment
SG	Specific Gravity
SSR	Simple-Sequence Repeat
TCT	Third Class Tuber
TTY	Total Tuber Yield

CHAPTER I

INTRODUCTION

The world’s population is expected to increase by 2 billion persons in the next 30 years, from 7.7 billion currently to 9.7 billion in 2050, and reach 10.9 billion in 2100 (Anon, 2020). This imperatively shows that the demand for food is bound to rise as well. With this demand for food products, also comes the burden on agronomists and plant breeders on how to produce high yield and high-quality crops, to avert food insecurity in the years ahead. In addition, the carbon footprint resulting from the rise in population is also negatively impacting the climate. This has led to the redefinition of the various agro-ecological zones around the world. It has also led to the emergence of numerous pathogens, which has accounted for various losses in trade stocks. The development of climate-resilient cultivars having resistance to main pests and diseases is crucial to combat these challenges related to population increase and climate change.

Potato (*Solanum tuberosum L.*) is the fourth most important food crop after wheat, maize, and rice in the world with annual global production of over 380 million tons, and the next most important food crop globally after rice and wheat in terms of consumption, based on humans (Anonymous, 2020). It is a very versatile crop utilized as raw material in the industrial, food, and feed has been highly recommended as a food security crop for the world`s growing population and related invidious problems with supply food (Devaux et al. 2014). However, potato is also very vulnerable to both abiotic and biotic stresses. Therefore, the development of new potato cultivars having high yield and quality potential as well as resistance to major diseases has a vital role in food sustainability. Hence, studies by potato breeders have seen the need to produce potato varieties that have high yield capacity and good quality, and it is also to get desirable varieties that has resistance PVY and PVX because potato virus X and potato virus Y are amongst the topmost imperative infectious viral diseases in worldwide.

Even controlling virus disease with chemical management is not possible because of the interactions between viruses and plant metabolism activities (Rubio et al., 2020). Sometimes is not so easy to differentiate viral disease symptoms. So, there are more than 50 different viruses and one viroid have been reported infecting worldwide potatoes (Jones, 2020; Kreuze et al., 2020; Rashid et al., 2021). Some of the potato viruses are potato virus Y (PVY), potato virus X (PVX), potato virus A (PVA), potato aucuba mosaic virus (PAMV), potato leafroll virus, (PLRV), potato virus S, (PVS), potato virus M (PVM), potato virus T (PVT) and potato virus B (PVB) Only a handful of them can cause high losses globally. On the other hand, some of the viruses are local while other viruses are a handful in the entire world. Potato leaf curling virus (PLCV), potato virus S (PVS), potato virus Y (PVY), and potato virus X (PVX) are reported that they cause severe infections worldwide (Hooker et al., 1988; Medina et al., 2017; Ahmed and Elci, 2019). Viral diseases are dispersal rapidly by mechanically, seedling tubers, workers, or by other vectors during the fieldwork of the year (Jones, 2006; Jeger, 2020; Rubio et al., 2020). To control viral diseases by chemical management is not honestly helpful (Basso et al., 2017; Wang et al., 2018). So, to get high yield and quality potato production, it is good to use potato seeds that are free from viral disease and it also uses potato varieties that have good resistance to viral diseases (Jones, 1988; Kreuze and Valkonen, 2017; Kreuze et al., 2020).

More than a billion people eat potatoes, and total global potato production exceeds 374 million metric tons per year (Tessema and Dagne, 2018). According to Food and Agriculture Organization (FAO) potato has been iterated and recommended as a food security crop produce as the world faces not only uncertainties in supply of food, but also steady hunger rates, a growing population, and massive increasing demand for food (Devaux, et al., 2014). It is a rich source of carbohydrates and has large amounts of protein, with a good quality of amino acid balance, vitamins A, vitamin B (B1 & B6), vitamin C, and also rich in carotenoids, flavonoids, caffeic acid (Ezekiel et al., 2013; Rajiv and Prashant, 2016). The dietary fiber of potato tuber is high, and potato skin contained a higher number of dietary fibres than flesh. Freshly harvested potatoes are virtually free of fat and cholesterol as compared to stored potatoes (Storey M. et al. 2007; Katherine, 2019). So, potato nutrients are very important and can be used to improved diets, thus reducing mortality rates caused by malnutrition. It can improve food security and health, especially among women and children (Kumara, M. et al, 2018). And have good properties of antioxidants, which help to maintain blood pressure, heart health, and low risks of stroke, brain function, nervous system coordination, and good for digestion. It is used in various ways such as French fries, chips, dehydrated potatoes, freshly used products, and alcohol production. To improve the livelihoods and food security of poor farmers, and it is also to get food for developing countries it is very important to increase in potato both yields and tuber quality. In this light, the importance of potatoes on the current global scale cannot be underestimated. Among the challenges of potato, breeding is the problems of low yield, low quality, and the mitigating forces of Potato viruses (PVY and PVX). Certification processes of a cultivar among others include its yielding capacity, tuber quality traits, and resistance to biotic stresses such as the potato viruses (PVY and PVX). Low-yielding potato varieties do not reflect the efforts of the breeders or potato producers and so results in production losses. Potato tuber quality parameters such as dry matter concentration, specific gravity, and French fries and chips quality are key determinants in the potato processing industries. The percentage dry matter and specific gravity of potato cultivars give prime information of its suitability for potato French fries and potato chips; thus, the potato tuber yield may fail quality standards and be unsuitable for the potato processing industries. High % dry matter concentration of ≥ 20 and specific gravity of ≥ 1.080 have been classified as ideal for potato French fries and chips processing and potato tubers with dry matter content and specific gravity lesser than their values are not ideal for French fries and chips processing (Haque et al. 2018). Potato virus infestations are also a great threat to potato breeders and affecting the actualization of food security. The occurrence of Potato virus Y (PVY) variant induces potato necrotic ring spot diseases (PTNRD) causing quality losses in the table and processed cultivars (Zimnoch-Guzowska et al. 2013), and a low rate (about 8 %) of seed certification and potato yield losses ranging from 10 to 90% resulting from decreasing harvestable potato tuber number and sizes (Zimnoch-Guzowska et al. 2013). The effects of PVY have resulted in the rejection of whole yields infestation. In addition, PVX infected plant’s field reduce its yield up to 40% but if it takes place at the same time with other viruses which belongs to the genus of Potyvirus such as PVY and PVA yield losses reach 80% (Kreuze, J. F. et al., 2020). İn this case, it depends on what type of viruses and what type of plant variety are planted Both of them potato virus y (PVY) and potato virus X (PVX) can cause a huge reduction of the yield production approximately between 50-60%, It is also is the most important viruses that can affect and contaminate seedling tubers. In addition, PVY and PVX viruses are accepted as two of the top 10 harmful plant viruses due to their adverse effects on crops (Scholthof, K.B.G, et al., 2011). İt is therefore highly important that high-yielding potato cultivars with high tuber quality and resistance to Potato virus Y and X are identified to boost the production and facilitate the certification of potato cultivars for human consumption. İn this regard, this research will identify high yielding potato cultivars with high tuber quality and resistance to PVY and PVX to increase potato production which meets industrial specifications

CHAPTER II

REVIEW OF LITERATURE

2.1 The importance of potato

Potato (*Solanum tuberosum*) is one of the most essential tuber crops in the world, is used for human and animal consumption, and is highly recognized as a source of food (FAO, 2009). Potato is one of the most widely grown crops in the world, adapting to various soil and weather conditions, with resultant high yield (Putz et al., 1990),
1989). Potato has been ranked as the third amongst imperative food security crops in the world as a whole after rice (*Oryza sativa L.*) and wheat (*Triticum aestivum L.*) (Haverkort et al., 2009), but its growth rate in production is the highest, as compared to other major food crops; thus, wheat, maize, and rice, due to its robust nutritional characteristics and high yield potential (Muhinyuza et al., 2016). However, in Turkey potatoes are fourth ranks after wheat, sugar beet, and barley (Caliskan et al., 2010). This has earned it its position as one of the country's sought-after agricultural food crops, due to its domestic use and industrial relevance.

Potato production is very important due to many reasons but the first important of potato are tuber quality, which includes biological (e.g., proteins, carbohydrates, and minerals) and sensorial traits (e.g., flavour, texture), and industrial traits (e.g., tuber shape, cold sweetening, and starch quality) (Kumara et al, 2018). The potato is a rich source of carbohydrates and has large amounts of protein, with a good quality of amino acid balance, vitamins C, vitamin B (B1 & B6). The dietary fibre of potato tuber is high, and potato skin contained a higher number of dietary fibres than flesh. Freshly harvested potatoes are virtually free of fat and cholesterol as compared to stored potatoes (Storey et al., 2007). So, potato nutrients are very important and can be used to improved diets, thus reducing mortality rates caused by malnutrition. It can improve food security and health, especially among women and children (Kumara. et al, 2018). To improve the livelihoods and food security of poor farmers, and it is also to get food for developing countries it is very important to increase in potato both yields and tuber quality.

2.2 History and origin of potato

Potato is derived from wild species *Solanum tuberosum* originates from the South America continent. Initially, it was first cultivated in Peru and Bolivia between 800 and 500 BC by the Inca indigents (Spooner et al., 2005), and its archaeological history date back to 2500BC (Harris et al., 2014). So, research identifies about 700 years before communities of hunters and gatherers, who had first entered South America, began cultivating wild potato plants that grew around the lake in abundance. Around 200 wild potatoes species are found in the Americas. At that time farmers, they got some challenge but they try to selecting and improving what was to become till to succeed.

The Spanish obtained the potato from Latin America and domesticated it in Europe in the 16th century. Adaptation to long days (Brown, 1990; Hawkes 1978, 1994) and generations of breeding led to a panel of potato varieties differing in taste, skin colour, shape, starch content, cooking type, etc. the significance of potato rose and distributed globally to its current regions with intensified cultivation and production. The fast-growing characteristic of potato allowed poor families to cultivate it on small plots and break the circle of poverty. Hundreds of millions of people around the world depend on the potato to survive. Potato is grown in more than 100 countries, under temperate, subtropical, and tropical conditions and ranks as the world's third most important food crop, after rice and wheat.

2.3 Biology of Potato

Potato (*Solanum tuberosum*) it is one of the Solanaceae families and herbaceous plant that grows up to 0.4 to 1.4 m tall and may rage from erect to wholly prostrate. Potatoes stems may be hairless or hairy and the colour my rage to purple, green, or stippled green and purple. Flowers are bisexual, hermaphroditic syncarpous, hypogynous, and different floral colours. Leaves are pinnate having a single terminal leaflet and three to four pairs of large ovoid leaflets with smaller ones in between (Struik, 2007). Potato is a self-pollination plant and can also reveal cross-pollination with generally green berry or capsulate fruits, mostly green with the axile type of placentation containing endospermic seeds of about 150 on average potato.

Rhizomes from the *S.tuberosum* plant known as stolons have undeveloped leaves and are usually hooked at the tip. They initiate from the basal stem nodes, normally below ground, with each node having up to three rhizomes (Struik, 2007). The shapes of the tubers are spherical to ovoid and about 20% dry matter and 80% water composition, the colour of fresh tubers are differs from white to yellow to blue and the skin varies from white to yellow and from red to blue. The texture of the surface may vary from smooth to netted (Spooner and Salas, 2006). The surfaces on the tuber are axillary buds with scars of scale leaves known as eyes (Struik, 2007). When tubers are sowed to the soil, the eyes develop into stems to form the next vegetative generation plant.

Potato cultivation takes four to nine months from sowing to harvest, due to differences among the genotypic makeup and origin (Maresma et al., 2019) requiring temperatures of 15 to 20˚C, pH of 4.8 to 8.5 for optimum yield and in diverse soil types leading to differences in maturation period. Potato is mostly cultivated on ridges to prevent tubers' exposure to light which makes the tubers green; an indication of increased glycoalkaloids and solanine levels, which are hazardous to human health (Chowański et al., 2016).

2.4 Taxonomic and Genetic Diversity of Potato

Potato (Solanum tuberosum L.) is a part of the Solanaceae family is about 3000–4000 species which include about 90 genera. Potato is a genetically different plant in the Solanum with both domesticated and wild types and it’s the largest genus of about 1500 to 2000 species (PBI Solanum Project 2014). And grouped into two tuber-bearing species (Petota section) and the non-tuber bearing species (Etuberosa). The tuber bearing is also sub-grouped into Potatoe and Estolonifera while Etuberosa and Juglandifolia are non-tuber bearing Hawkes (1990) Spooner et al. (2007) indicated that 141 infra-specific taxa of potato exist within the cultivated potato germplasm. (Spooner et al., 2016). added the S. tuberosum as a member of the tuber-bearing potatoes and reported that, 232 wild species. The wide variation in the wild species in the gene pool and distribution is an indication of high tolerance to biotic and abiotic stresses (Machida-Hirano, 2015). In addition, 288 different species of potato were identified using SSR and AFLP techniques which indicated that have high genetic diversity with different levels of biotic and abiotic stresses resistance and adaptabilities (Wang et al. 2017)

Several taxonomic classifications of potato had been given which are blamed on interspecific hybridization, sexual and asexual reproduction, species divergence, auto- or allopolyploidy, phenotypic plasticity, high morphological similarity among species among others (Machida-Hirano, 2015). Stated that cultivated potato varieties are either landraces, native varieties, or improved varieties with a variety of tuber shapes, skin, and flesh colours (CIP, 2014) and grow within elevations of 3000–4000m above sea level.

Potato is cytologically diploid (2n = 2x = 24), triploid (2n = 3x = 36), tetraploid (2n = 4x = 48), pentaploid (2n = 5x = 60) or hexaploid (2n=6x=72) with a fundamental chromosome number of 12 (Gavrilenko, 2007). While the diploid, tetraploid, and allohexaploids are sexually fertile, the triploid and pentaploid are sexually sterile reproduced by vegetative propagation. About 75% of the Solanum tuberosum species are diploids which and self-incompatible, the tetraploid 15% and the remaining are self-compatible, express inbreeding depression and male sterility (Watanabe, 2015).

2.5 Viruses of Potato

Viruses are somewhat small organisms that have RNA or DNA encoding the viral genome surrounded by a protein coat and they cannot be observed even microscopes. Besides viruses themselves to grow need living organism for this purpose, they transfer among living organisms with vector. Unfortunately, because of increasing temperature possible numbers of virus vector progressively grows and the occurrence of epidemics that originated from viruses arise (Kreuze et al., 2020) Management of viruses depends on their persistence and viruses can be semi-persistently, persistently, or non-persistently vector transmitted. Semi-persistently or persistently vector transmitted viruses (e.g., PVX) may control by insecticide application as tuber dressing or foliar sprays during the seed potato production process. However, non-persistently vector-borne transmitted viruses like PVY that innately transmitted by mechanically or more than 40 aphid species, so they do not control with these applications (Jones, 2014; Kerlan et al., 2006; Radcliffe and Ragsdalee, 2002). Viruses are very problematic and it also very difficult to control, for potato production there are more than 50 different viruse and one viroid has been reported infecting worldwide potatoes (Jones, 2006). Some of the handful of them can cause huge losses worldwide, while some are locally and minor crucial anywhere on the globe. The most important potato viruses are PVY (Table 2.1) and PLRV and the most damaging viruses of potatoes globally. The invidious losses of tuber yield caused by any of viral agents' infections may attain more than 80% associated with other infectious viral microbes.

PVX emerges normally and invidiously causing losses of about 10–40% in a single infectious disease which is most devastating in association with PVY or PVA. This is due to the synergistic association with *potyviruses* towards losses in tuber yield of up to about 80%. PVS also emerges commonly on the globe, but generically causing only minor tuber yield losses without presenting invidious strains or it occurs in diverse infectious diseases with PVX. PVA has the potential to cause devastating damage in yield of up to about 40% by itself but is wide less hugely spread than PVY, PVS, or PLRV. PVM is relatively uncommonly in most geographical areas as PVS, mostly cause only minor losses in tuber yield, except in associated infections with PVX or other infectious viral microbes.

2.6 Potato Virus (PVY and PVX)

2.6.1 Potato virus Y (PVY)

Potato virus Y (PVY) is a virus species in the genus Potyvirus. The most important strains of PVY are PVY^{O}, PVY^{N,} and PVY^{C} (Upeksha et al., 2012). PVY^{O} is the familiar strain virus causing mosaic symptoms while PVY^{C} causes stipple streak, and PVY^{N} is a necrotic strain that usually causes mild foliage symptoms (Whitworth et al., 2012). Different mixed infection strains generally produce hybrid strains so PVY^{NO} and PVY^{NT} (Beczner et al., 1984; Singh et al., 2008; Whitworth et al., 2012)

Potato virus Y (PVY) is the most handful of viral pathogens that causes infection potato plant (Kogovšek et al., 2008). In 1931 PVY was recognized as a member of the virus strain group related to the deterioration potato plant (Smith, 1931). Genus Potyvirus is the biggest and most important group of known plant viruses and can cause destructive diseases in many cultivated plants like most Solanaceae or nightshades family-like tobacco, tomato, potato, and pepper (Hollings and Brunt, 1981). PVY has very large host plants and extends around the world (Lorenzen et al. (2006) reported an infection of approximately nine families of Solanaceae, including 14 genera. Hosts include tomatoes (*Lycopersicum esculentum*), pepper (*Capsicum* spp.), Eggplant (*S. melongena*), tobacco (*Nicotiana spp*), and many herbs (*Physalis* spp., *Datura* spp., *S. dulcamara*, and *S. nigrum*) (Jeffries, 1998). The experimentally reported host range consists of 72 genera and 495 species of 31 families. About 287 species of Solanaceae in 14 genera, including 141 species of Solanum, 70 species of Nicotiana, 28 Amaranthaceae, 25 Leguminosae, 20 Chenopodiaceae, and 11 Compositae species were reported as hosts (Binyam, 2015). PVY has a huge problem for the production of potato plant because have a massive impact on the reduction of the yield and it also can spread very fast and easily. Aphids are the most important PVY potato virus vector specially *Myzus persicae* which transmit the virus in a non-persistent manner (Ragsdale et al., 2001). It is also PVY can transmit mechanically by machinery, tools, or workers when they are working or walking for the field or from the mother plant through infected tubers (Salazar, 1996). PVY is one of the leading causes of the rejection of seeds in certification programs (Ragsdale et al., 2001). The losses caused by PVY are 10-80% depending on the variety (Warren et al., 2005). However, some PVY races may cause a 10- 100% loss of yield in potato production (De Bokx and Huttinga, 1981). To get PVY resistance potato genotype there are four genes which cause completely defend against PVY are Ry$_{adg}$, Ry$_{sto}$, Ry-f$_{sto}$, Ry$_{chc}$. These four genes' chromosomal location level and their origination are obtained (Table 2.2) (Ohki, 2018). In addition, extreme resistance genes are different, so this study used extreme resistance based on Ry$_{sto}$ and Ry$_{adg}$ genes. These two genes have the 11th chromosome and the position they will appear in is the same (Hamalainen et al., 2000).

Table 2.1. Potato extreme resistance genes against PVY

Extreme Resistance genes	Origin	Chromosome
Ryadg	*Solanum tuberosum* subsp. *andigena*	11
Rysto	*Solanum stoloniferum*	11
Ry-fsto	*Solanum stoloniferum*	12
Rychc	*Solanum chacoense*	9

Hypersensitivity gene to Potato virus Y, Hypersensitivity is controlled by single dominant genes and for arising hypersensitivity ‘‘gene for gene’’ phenomena required. The concept gene for the gene was discovered by Flor (1946) he mentioned in his explanation the relationship between pathogen and host in terms of resistance (R) gene and avirulence (Avr) genes. To be the functional genome of pathogen resistance (R) gene need to avirulence (Avr) genes (Hammond-Kosack, K.E., et al., 1994). Infected plant’s R genes noticed an elicitor (a chemical compound that causes a hypersensitive response) which segregated from Avr gene of pathogen and the process provide resistance as a response from the plant (Heitefuss, R. and Williams, P.H 2012). So, there are three hypersensitive genes as Nctbr, Nztbr, and Nytbr (Karasav A.V. et al., 2011) three of them are originated from Solanum tuberosum. Nctbr is responsible to elicit a hypersensitive response to PVYC and Nytbr causes a hypersensitive response against PVYO strain, (Tian, Y.P. et al., 2013). Nytbr and Nctbr genes cannot demonstrate hypersensitive reaction against some PVY strain groups so the researchers called as part of Nztbr (Singh, R.P. et al., 2008) It elicits the response against PVYZ (Table 2.3) is the overall mechanism of HP in potato.

Table 2.2. Interaction among the PVY strains and hypersensitivity genes

PVY Hypersensitivity Genes Based on Strains				
PVY Strain Groups	PVY^{O}	PVY^{C}	PVY^{N}	PVY^{Z}
Genes show HP	Ny$_{tbr}$	Nc$_{tbr}$	No HP response	NZ$_{tbr}$

2.6.2 Potato Virus X (PVX)

Potato virus X (PVX) is one of the main potato viruses which cause infection in all countries where potato cultivation is done (Gebhard et al., 2006). PVX belongs to the genus Potexvirus and group 14 is the main strain group of PVX (Cockerham, 1955). The other PVX stain is the resistance-breaking strain PVX^{HB} (Moreira et al., 1980) and the more recently described PVX ^{OS} (Kagiwada et al., 2002) and PVX Tula (Ravine et al., 2008). PVX is not only transmitted by an insect vector, but also the virus is often transmitted mechanically (Salazar, et al., 1996). The speed of PVX is quid and rapid from sowing to harvest, from infected plant to health plant by agricultural machinery, from diseased plant to health plant by the mouth part of the insects (Hooker, 1986). Symptoms of PVX are decreased leaf size, chlorosis, mosaic and necrotic lesions in tubers (Salazar et al., 1996). The loss caused by the PVX virus is 5 to 20% of reduction tuber yields. When PVX is met with other viruses like PVY and PVA can cause more symptoms and reduction yield than other viruses alone (Jayashige et al., 1989). Mixed infection of potato plants with PVX and PVS also can cause tuber yield losses when none occur with either virus alone (Wright, et al., 1977). Chemical control is not suitable to control virus disease; resistance is the most actual method to control this virus. Dominant resistance gene Rx1 and Rx2 was determined in Solanum tuberosum subsp. Andigena (Ahmadvand et al., 2013). Ohbayashi et al. (2010) Discovery of the genes was respectively achieved by map-based cloning and Agrobacterium transient expression methods (Bendahmane et al., 1999, 2000). Both genes are placed in different chromosome positions, Rx1 takes place on the 12th chromosome, Rx2 is rooted on the 5th chromosome (Bendahmane et al., 2000). Rx1 gene derived from Solanum tuberosum ssp, Whereas Rx2 genes are derived from Solanum acaule (Shaikhaldein et al., 2018). In addition, Rx1 and Rx2 genes are so similar based on nucleotide sequence and amino acid sequence 98% and 96% respectively (Ahmadvand R. et al., 2013; Bendahmane et al., 2000). These two genes work as homologous and they identify identical Avr genes (de Ronde D. et al., 2014).

Hypersensitivity genes to PVX strains. Four different PVX strains infect plants. The effects of the strains are different according to the hypersensitivity genes Nx and Nb. The First strain doesn’t defeat both Nx and Nb, the second strain cannot beat Nb, but it beat Nx, the third one disables Nb and the last strain can overcome both genes (Kutnjak et al., 2014). It also reckoned out the interaction of hypersensitivity genes and PVX strains (Table 2.4). HP genes whether combat with the viruses or not combat up to strain type. However, N resistance genes (ER) always defeat the virus attack and the genes symbolize RX.

Table 2.3. Interaction among the PVX strain and hypersensitivity genes

PVX Hypersensitivity Genes Based on Strains				
PVX Strain Groups	X_1	X_2	X_3	X_4
Gene/s shows HP	Nx and Nb	Nb	Nx	No HP response by genes.

CHAPTER III

MATERIALS & METHODS

3.1 Plants materials

Twenty-eight potato breeding lines were developed at the Agricultural Genetic Engineering Department, Niğde Ömer Halisdemir University, and five commercial cultivars (Agria, Alegria, Lady Olympia, Marabel, and Melody) as standard cultivars were evaluated for yield and quality parameters under field conditions in Nigde. All breeding lines and cultivars were also being screened with molecular markers (SSR) for PVY and PVX resistance. The list of potato breeding lines and cultivars, which were used in the thesis, is given in Table 3.1.

Table 3.1. The list of potato breeding lines and cultivars

No	Code	Parents (♀ x ♂)
1	17009.01	Agria x Bafana
2	17009.02	Agria x Bafana
3	17011.01	Agria x CIP397055.2
4	17011.03	Agria x CIP397055.2
5	17011.11	Agria x CIP397055.2
6	17011.13	Agria x CIP397055.2
7	17011.15	Agria x CIP397055.2
8	17011.17	Agria x CIP397055.2
9	17011.18	Agria x CIP397055.2
10	17013.03	Agria x Galata
11	17023.01	Bafana x Pomqueen
12	17023.02	Bafana x Pomqueen
13	17023.03	Bafana x Pomqueen
14	17023.04	Bafana x Pomqueen
15	17024.02	Bafana x Tessa
16	17053.04	Compass x Alegria
17	17065.02	Hermes x Bafana
18	17075.01	Lady Olympia x Bafana
19	17075.08	Lady Olympia x Bafana
20	17076.02	Lady Olympia x GH113
21	17076.03	Lady Olympia x GH113
22	17078.03	Lady Olympia x Navigator
23	17078.06	Lady Olympia x Navigator
24	17080.01	Lady Olympia x Rumba
25	17080.06	Lady Olympia x Rumba
26	17080.14	Lady Olympia x Rumba
27	17084.01	Nautilus x GH113
28	17092.03	Navigator x Galata
29	Agria	Quarta x Semlo
30	Alegria	Divina x 3.169 010-86
31	L.Olympia	Agria x KW 78-34-470
32	Marabel	Nena x MA 75 364
33	Melody	VE7445 x W72.22.496

3.2 Experimental method

3.2.1 Site selection and location

The study was conducted in Niğde, from April to October in 2020. Niğde lies within 37.9698° N, 34.6766° E, the altitude in Niğde is 1244m and the annual average temperature is 10.3 °C, it also averages precipitation in Niğde is 338mm. The monthly temperature and rainfall of this study during the research period are presented in Table 3.2.

Table 3.2. Monthly mean temperature and rainfall Nigde in 2020

	Location	may	June	July	August	September	October
Mean Temperature (^oC) (1981-2010)	Niğde	15.3	19.7	22.9	22.7	18.2	12.4
Mean Temperature (^oC) (2020)	Niğde	17.6	26.7	22.2	22.6	18.2	15.1
Rainfall (mm) (1981-2010)	Niğde	46.4	24.5	5.7	5.3	7.6	30.4
Rainfall (mm) (2020)	Niğde	26.2	43.4	8.3	6.0	6.0	13.7

Source: Turkish State Meteorological Service

3.2.2 Experimental design and setup

The field experiment was layout in a Randomized Complete Block Design with four replications. Each plot was consisted of two rows having 8.1 m in length and 70 cm apart. The experiment was planted on 17/05/2020. The seed tubers were planted using a two-row planter with 30 cm in-row and planting distance of 54 tuber seeds per plot, each row with 27 tubers. All the plots were fertilized with 100kg/ha N-P-K and 200kg/ha urea. Before planting the potatooes seed was treated with a fungicide (active ingredient: 100 g/l Penflufen+18 g/l Prothioconazole) at a 20 ml/100kg seed rate, at the same time insecticide was given before planting at the rate (active ingredient: 350g Thiamethoxam) at 20 ml/100 kg seed rate. The irrigation of the experiment was overhead sprinkler irrigation and to maintain the weed-free from the field, hand weeding was done during the growing period.

The harvesting date of the experiment was 25/09/2020 using a two-row harvesting machine. After harvesting, the potato tubers were collected in bags per plot and labeled with the block and plot number, after that the tubers were graded, weighed, and the dry matter and specific gravity analyses were done.

3.3 Evaluated Traits

Stand establishment (%): The number of plants in each plot was counted at the flowering stage and the emergence rate was calculated by dividing the plants germinated over the number of seed tuber sowed multiplied by hundred.

Number of stems per plant: The number of main stems per plant was counted on ten randomly selected plants in the plot at the flowering stage.

Number of tubers per plant: All tubers in each plot were counted and numbers of tubers per plant were calculated by dividing total plants in the plot.

Mean tuber weight (g): Mean tuber weight was calculated by dividing total tuber weight by total tuber number in each plot.

Tuber size grading (%): The tubers of each genotype were separated into three grades: cull (<30 mm or damaged), the second class (30-50 mm in diameter), and the first-class (>50 mm), and proportion of each grade in total weight were calculated.

Total tuber yield (t/ha): Total yield of each genotype was calculated by weighing all tubers in each plot. Therefore, the yield per plot was determined, the plot yield was converted to the yield in ton per hectare.

Marketable tuber yield (t/ha): The marketable tubers (>30 mm) in each plot were weighed to identify marketable tuber yield per plot. Then, marketable tuber yield per plot was converted to marketable tuber yield in ton per hectare**.**

Specific gravity and dry matter content (%) analyses:

The specific gravity and dry matter content of potato tubers were measured using Martin Lishman PW2050 digital hydrometer (Figure 3.1) with weighing around 2 kg potato tubers under water and above water.

Figure 3.1. Martin Lishmans's digital potato hydrometer (PW2050) was used to measure specific gravity and dry matter content of potato tubers

3.4 Statistical Analysis

The data were analysed statistically using the analysis of variance (ANOVA) with the help of a computer using the MSTAT-C program (Gomez and Gomez, 1984). Statistix statistical software (version 8.1) was used to perform the mean comparison test of Least Significant Difference (LSD) Tests at a 5% level of significance.

3.5 Screening of Genotypes with Molecular Markers

3.5.1 Collection and preparation of leaf samples for DNA isolation

Twenty-eight potato breeding lines and five commercial cultivars in the experiment were evaluated to determine the resistance to PVY and PVX within the scope of the thesis. DNA extraction was performed for each of the sample and CTAB method was preferred. Moreover, DNA extraction protocol followed from instruction of Doyle (1991). Before starting to DNA extraction, freshly collected leaf samples were weighed approximately 0.2 g into 2 ml Eppendorf tubes. Together with the leaf samples and 2 pieces of small balls are placed in each Eppendorf tube. After that, all the samples were run by a TissueLyser device at a 28Hz-frequency speed for 5 minutes. At the same time, the crushed young leaves in Eppendorf tubes were stored at -80°C for one day.

3.5.2.DNA extraction

CTAB extraction solution content and ratios to expose DNA are given in Table 3.3

Table 3.3. CTAB buffer solution content

Stock	Quantity for 500 ml	Final Concentration
1 M Tris pH 8	50 ml	100 Mm
0.5 M EDTA pH 8	20 ml	20 Mm
4 M NaCl	175 ml	1.4 M
CTAB	10 gram	2%
PVP 40	10 gram	2%
Na_2S2O5	500 mg	0.1%

The solution takes 12 hours for CTAB and PVP to dissolve homogeneously in the solution. After thorough mixing the solution was autoclaved to a homogeneous state, then the kept at 4°C to be used in DNA isolation.

1 M Tris pH 8.0 (1litter)

121.1 grams of Trisma base was weighed and added to 800 ml distilled water. Stock bottles with the help of a measuring tape then the solution was mixed in the stock bottle. The pH of the solution was measured with probes and HCl was administered in a controlled manner to adjust the pH of the solution to 8.0. Then the final volume obtained was made up of 1 litre of pure water and the solution was autoclaved**.**

0.5 M EDTA pH 8.0 (1 litre) and 4 M NaCl

186.1 grams of EDTA was weighed and add 800 ml of distilled water and the pH of the solution measure. The pH of the solution was adjusted to 8.0 with 10M NaOH. After the final volume of solution is made up of 1 litter and the EDTA is completely dissolved that the solution was autoclaved.

For 4 M NaCl,

243.3 grams of NaCl was added to 800 ml of distilled water and mixed them. After completion, the total volume was made up to 1 litter with distilled water.

Preparation of TBE Buffer (1 L):

To prepare 1000 ml of TBE Buffer stock solution, 54-gram Tris Base, 27.5 grams Boric Acid, 200 ml of 0.5M EDTA was added, to make 1000ml about 800 ml of distilled water is added until EDTA can dissolve. So, the solution was mixed and dissolved homogeneously. The prepared Stock solution is 5X density and 0.5X to be used in agarose gel making an electrophoresis tank. The steps of DNA extraction are given in Table 3.4

Table 3.4. Genomic DNA isolation

1	Set Biosan Thermo-Shaker temperature 65 °C
2	2 steel balls were placed on microtubes with leaf samples and the leaf sample was powdered with QIAGEN tissue lyser 2 during 5-10 minutes. Frequency adjusted to 28.
3	900 ul CTAB buffer adding into 2 ml microtubes.
4	Mix solution with ISOLAB vortex.
5	12 ml microtubes incubate at 65 °C for 1 hour using Biosan Thermo-Shaker and every 10 minutes microtubes shake with the shaker.
6	After 1-hour incubation, keep the sample at room temperature for 5 minutes.
7	To add 900 µl chloroform/isoamyl alcohol (24:1).
8	Gently up down microtubes for 15 minutes.
9	Centrifuge the sample for 15 minutes at 14.000 rpm with Sigma 3-30KS.
10	Transfer upper phase (almost 700 µl) to 1.5 ml microtube.
11	Add 500 µl cold isopropanol and incubate at -20 to provide DNA precipitation.
12	Centrifuge sample for 5 seconds at 10.000 rpm using Sigma 1-14K centrifuge and then to prevent falling pellet from microtubes when removing the supernatant.
13	To add 76% ethanol this includes 1ml 100 mM Sodium acetate into microtubes.
14	Gently up down microtubes for 15 minutes again.
15	Centrifuge sample for 5 seconds at 7.000 rpm using Sigma 1-14K centrifuge and remove upper aqueous phase.
16	Dry pellet to clean alcohol and add 50 µl ddH2O.

3.5.3 Analysis of the quantity and quality of DNA samples

DNA quantity and purity analysis by spectrophotometer

1 ul liquid dsDNA sample was measured with spectrophotometer. For measurement, firstly 1 ul ddH_2O was loaded upper surface of lower arm and then pushed blank button to reset. After that DNA sample of each genotype was measured 2 times and their average calculated. Dilutions of them calculated with the help of M1*V1=M2*V2 formula. Furthermore, 50ng/ul DNA was diluted to10 ng/ul to be proper for PCR.

Preparation of 1% agarose Gel for loading DNA samples:

TBE Buffer diluted to 0.5 X density was measured with the help of a measuring tape; 120 ml are taken into the temperature resistant beaker which was used to prepare the gel. On TBE buffer 1.2grams of agarose was added and then the solution was moved in the microwave oven for 3 minutes of heating until it was dissolved and turns transparent. After that, the solution flowing out of the microwave and then was cooled under tap water, and then 5 µl EtBr (10 mg/ml) was added and allowed to mix into the solution. The beaker was shaken gently, taking care not to form air bubbles at this stage show.

Imaging genomic DNA by electrophoresis:

While the prepared gel is in the liquid phase, it is gently placed on the gel tray of the electrophoresis device from one corner. Care was taken to avoid the formation of spilled air bubbles. DNA in gel the comb is placed to create the wells where the samples will be loaded, and the gel is transferred to the solid phase. The gel was incubated at room temperature for 30 min. Comb from the solidified gel was removed by holding it from both parts and taking care not to tear the wells. The tray was placed in an electrophoresis tank filled with 0.5X TBE buffer. 5µl DNA and 3µl loading dye were loaded in sample wells. Run time and voltage were adjusted for 1 hour/105 V. Afterwards, the DNA gel result was checked.

Dilution of DNA samples according to the PCR protocol to be screened

The isolated DNA samples were determined according to the DNA densities in the previous protocols. DNA samples for the PVY PCR protocol at a density of 5ng/µl while for PVX PCR protocol for the DNA density was diluted to 6.5ng/µl. Samples using data from Nano drop measurements for this formula “M1.V1 = M2.V2” were calculated.

Polymerase chain reaction (PCR):

Extracted DNA from each of the 33-plant samples ware scanned for 1RX1 and 106Rx2 primers for determination of resistance to PVX and STM0003 primer for PVY. The sequences and the amplicon sizes of the primers used are given in Table 3.5.

Table 3.5. Molecular marker primer sequences

Marker	Sequences	Target Gene	Amplicon size (bp)	Virus	Reference
1Rx1	5’- GGAGAAATCCTGCAATATAAT-3’ 3'- CGACCGAACTTACATTTTCCC-5’	Rx1	974	PVX	Ritter et al. 1991
106Rx2	5’-GGAGAAATCCTGCAATGTAAC-3' 3'-CTTGTCAAAGAAAGAAGGCCT-5’	Rx2	543	PVX	Ritter et al. 1991
STM0003	5’-GGGGCAGGATGCGTTTTTGAG-3' 3'-AATTGTAACTTGTTGTGTGTG-5’	Rysto	111-140	PVY	Lopez et al. 2015

Information about PCR primers, Denaturation, annealing temperature, and extension condition was listed in Table 3.6.

Table 3.6. PCR conditions for PVX condition

1RX1 AND 106RX2		PCR STEPS for 1Rx1 and 106Rx2		
DNA (6.5ng/µl)	6.5µL	Initial Denaturation	94 °C /3 minute	
10X Dream Taq Buffer ($MgCl_2$)	2.0 µL	Denaturation	94 °C/30 seconds	30 cycles
DNTPs(10 mM each)	0.5 µl	Annealing	50 °C/45 seconds	
5Rx1_F (5µM)	0.5µl	Extension	72 °C/ 45 seconds	
5Rx1_R (5µM)	0.5 µl	Final Extension	72 °C/ 10 minutes	
106Rx2_F (5µM)	3.0 µl		+4 °C/∞	
106Rx2_R (5µM)	3.0 µl			
DNA Taq Polymerase (5µM)	0.2 µL			
dH$_2$0	3.8 µL			
Total	20.0 µl	Product Size	974 and 543 bp	

Table 3.7. PCR Conditions for PVY

STM 0003		PCR STEPS		
DNA (5ng/µl)	15 µL	Initial Denaturation	94 °C /3 minute	
10X Dream Taq Buffer ($MgCl_2$)	2.5 µL	Denaturation	94 °C/30 seconds	40 cycles
dNTPs (10 mM each)	1.0 µl	Annealing	50 °C/45 seconds	
STM0003 Forward Primer (5µM)	1.5 µl	Extension	72 °C/ 45 seconds	
STM0003 Reverse primer (5µM)	1.5 µl	Final Extension	72 °C/ 10 minutes	
DNA Taq Polymerase (5µM)	0.3 µl		+4 °C/∞	
dH$_2$0	3.2 µl			
Total	25.0 µl	Product Size	111 bp	

Figure 3.2. Checking DNA for Spectrophotometry

Figure 3.3. Putting DNA samples in tank

Figure 3.4. Putting samples into the PCR

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Stand Establishment (%)

The results of the ANOVA of the percentage stand establishment of the potato genotypes showed significant differences among the genotypes (Table 4.1). As presented in Table 4.2, the stand establishment ranges from 92.13 to 99.54% with the average stand establishment of the genotypes being 97.06%. The highest emergence of 99.54% was recorded in genotypes MEÇ17011.11, MEÇ17011.17, MEÇ17023.02, and MEÇ17080.14, while the lowest establishment 92.13% was recorded in MEÇ17078.03. This difference in the stand establishment of the genotypes is due to differences in genotypic origin and it is also decaying of some seed tubers after planting due to disease, and it is also soil condition especially when irrigation caused water standing part of the field. These results agree with the findings of Alva et al. (2002).

Table 4.1. Analysis of variance of stand establishment of potato genotypes grown in Nigde

Source	DF	SS	MS	FV	P-value
Replication	3	30.355	10.118	1.1978	0.3149 ns
Genotype	32	462.470	14.452	1.7108	0.0240*
Error	96	810.961	8.448		
Total	131	1303.786			
CV%	2.99				

DF= degree of freedom, SS= sum of square, MS= mean square, CV= coefficient of variation FV= F value, *p≦0.05, ns= not significant

Table 4.2. Average values of stand establishment of potato genotypes grown in Nigde.

Treatment	% Stand Establishment (SE)	Group Letter*
AGRIA	95.83	ABCDE
ALEGRIA	96.76	ABCD
L.OLYMPIA	94.97	BCDE
MARABEL	97.22	ABCD
MELODY	94.48	CDE
MEÇ17009.01	98.14	ABC
MEÇ17009.02	98.61	AB
MEÇ17011.01	96.76	ABCD
MEÇ17011.03	97.22	ABCD
MEÇ17011.11	99.54	A
MEÇ17011.13	97.68	ABCD
MEÇ17011.15	95.83	ABCDE
MEÇ17011.17	99.54	A
MEÇ17011.18	97.22	ABCD
MEÇ17013.03	98.61	AB
MEÇ17023.01	98.60	AB
MEÇ17023.02	99.54	A
MEÇ17023.03	97.68	ABCD
MEÇ17023.04	97.68	ABCD
MEÇ17024.02	98.15	ABC
MEÇ17053.04	98.61	AB
MEÇ17065.02	98.15	ABC
MEÇ17075.01	96.75	ABCD
MEÇ17075.08	97.68	ABCD
MEÇ17076.02	97.22	ABCD
MEÇ17076.03	92.13	E
MEÇ17078.03	98.14	ABC
MEÇ17078.06	92.59	E
MEÇ17078.03	96.75	ABCD
MEÇ17080.06	97.68	ABCD
MEÇ17080.14	99.54	A
MEÇ17084.01	93.98	DE
MEÇ17092.03	96.75	ABCD
Mean value	97.06	

*Different letters within column indicate significance at p≤0.05 LSD test

4.2 Number of Stems per Plant

In Table 4.3, the ANOVA results showed that there was no-significant difference in the number of stems per plant (NSP) among the genotypes. The number of stems per plant ranged from 3.88 (MEÇ17011.18) to 4.80 (MEÇ17009.01) with the average number of stems per plant being 4.28 (Table 4.4). This shows that there is no genotypic variability among the genotypes in terms of the number of stems (Table 4.3).

Table 4.3. Analysis of variance for stem numper of potato genotypes grown in Nigde

Source	DF	SS	MS	FV	P-value
Replication	3	0.305	0.102	0.3870	0.002**
Genotype	32	9.119	0.285	1.0844	0.3709ns
Error	96	25.229	0.263		
Total	131	34.653			
CV%	11.96				

DF= degree of freedom, SS= sum of square, MS= mean square, CV= coefficient of variation, FV= F value, ns= not significant

Table 4.4. Average values of stems per plant of potato genotypes grown in Nigde

Treatment	No of Stems per Plant	Group Letter*
AGRIA	4.30	ABCDE
ALEGRIA	4.05	BCDE
L.OLYMPIA	4.55	ABCDE
MARABEL	3.95	DE
MELODY	3.90	DE
MEÇ17009.01	4.80	A
MEÇ17009.02	4.75	AB
MEÇ17011.01	4.45	ABCDE
MEÇ17011.03	4.70	ABC
MEÇ17011.11	4.60	ABCD
MEÇ17011.13	4.70	ABC
MEÇ17011.15	4.75	AB
MEÇ17011.17	4.30	ABCDE
MEÇ17011.18	3.88	E
MEÇ17013.03	4.15	ABCDE
MEÇ17023.01	4.45	ABCDE
MEÇ17023.02	4.35	ABCDE
MEÇ17023.03	4.25	ABCDE
MEÇ17023.04	4.35	ABCDE
MEÇ17024.02	3.85	E
MEÇ17053.04	4.30	ABCDE
MEÇ17065.02	4.25	ABCDE
MEÇ17075.01	4.10	ABCDE
MEÇ17075.08	4.10	ABCDE
MEÇ17076.02	4.00	CDE
MEÇ17076.03	4.13	ABCDE
MEÇ17078.03	4.15	ABCDE
MEÇ17078.06	4.23	ABCDE
MEÇ17080.01	4.31	ABCDE
MEÇ17080.06	4.13	ABCDE
MEÇ17080.14	4.01	CDE
MEÇ17084.01	4.40	ABCDE
MEÇ17092.03	4.25	ABCDE
Mean value	4.28	

*Different letters within column indicate significance at p≤0.05 LSD test

4.3 Number of Tubers per Plant

The result of the analysis of variance for the number of tubers per plant showed that both genotypes and replication have a highly significant (P< 0.001) effect on the number of tubers per plant of the potato genotypes with a CV at 12.05% (Table 4.5). Table 4.6 presents the mean number of tubers per plant of the potato genotypes. The number of tubers per plant ranges from MARABEL (4.15) to MEÇ17078.06 (13.05) with an average tuber number of 7.33 with significant differences occurring across the lowest to the highest number of tubers per plant which shows different lettering by the LSD test (Table 4.6). The difference in the number of tubers per plant might be dependent on the genotypic origin of the genotypes and their responses to environmental conditions. This result confirmed the findings of Khan et al. (2018) and Eaton et al. (2017) that the number of potato tubers depends on the environmental condition and genomic origin.

Table 4.5. Analysis of variance for the number of tubers per plant of potato genotypes grown in Nigde

Source	DF	SS	MS	FV	P-value
Replication	3	0.149	0.050	0.0282	0.0000**
Genotype	32	445.985	13.937	7.9411	0.0000**
Error	96	168.484	1.755		
Total	131	614.617			
CV%	12.05				

DF= degree of freedom, SS= sum of square, MS= mean square, CV= coefficient of variation FV= F value, **= high significant,

Table 4.6. Average values of number of tubers per plant of potato genotypes grown in Nigde

Treatment	Number of Tubers per Plant	Group Letter *
AGRIA	8.80	BCDEFG
ALEGRIA	7.07	FGHIJKL
L.OLYMPIA	9.55	BCD
MARABEL	4.15	O
MELODY	7.80	DEFGHI
MEÇ17009.01	6.52	IJKLM
MEÇ17009.02	4.40	NO
MEÇ17011.01	6.67	HIJKLM
MEÇ17011.03	9.97	B
MEÇ17011.11	9.77	BC
MEÇ17011.13	8.00	CDEFGHI
MEÇ17011.15	7.13	FGHIJKL
MEÇ17011.17	9.15	BCDE
MEÇ17011.18	9.73	BC
MEÇ17013.03	7.65	EFGHIJ
MEÇ17023.01	5.45	LMNO
MEÇ17023.02	5.15	MNO
MEÇ17023.03	5.90	JKLMNO
MEÇ17023.04	6.78	HIJKLM
MEÇ17024.02	6.40	IJKLM
MEÇ17053.04	6.38	IJKLM
MEÇ17065.02	5.08	MNO
MEÇ17075.01	6.15	IJKLMN
MEÇ17075.08	7.70	DEFGHIJ
MEÇ17076.02	7.25	EFGHIJK
MEÇ17076.03	7.15	FGHIJKL
MEÇ17078.03	7.02	GHIJKL
MEÇ17078.06	13.05	A
MEÇ17080.01	8.45	BCDEFGH
MEÇ17080.06	8.90	BCDEF
MEÇ17080.14	7.45	EFGHIJK
MEÇ17084.01	5.77	KLMNO
MEÇ17092.03	5.42	LMNO
Mean value	7.33	

*Different letters within column indicate significance at $p \leq 0.05$ LSD test

4.4 Mean Tuber Weight (g/tuber)

The ANOVA results revealed highly significant (P< 0.001) differences of the mean tuber weight among the genotypes, that occurs at CV of 3.89%. The mean tuber weight of the genotypes ranged from 65.64g to 138.06g with an average tuber weight of 91.64g (Table 4.8). The highest mean tuber weight (g) was revealed in MEÇ17011.15 (138.06) whereas the lowest mean tuber weight was found in MEÇ17084.01 (65.64). The dissimilarity in mean tuber weight depends on the inheritability of genotypes. Different varieties may Couse different traits (Kumar et al., 2004). The weight of the tubers may Couse rapid germination and well plant growth (Patel et al., 2008).

Table 4.7. Analysis of variance of mean tuber weight gram of potato genotypes grown in Nigde

Source	DF	SS	MS	FV	P-value
Replication	3	25.728	8.576	0.6739	0.0000**
Genotype	32	48792.172	1524.755	119.8248	0.0000**
Error	96	1221.588	12.725		
Total	131	50039.488			
CV%	3.89				

DF= degree of freedom, SS= sum of square, MS= mean square, CV= coefficient of variation FV= F value, **= high significant

Table 4.8. The average values of mean tuber weight of potato genotypes growing in Nigde

Treatment	Mean tuber weight (g/tuber)	Group Letter*
AGRIA	83.22	IJ
ALEGRIA	101.30	FG
L.OLYMPIA	84.23	IJ
MARABEL	81.06	IJKL
MELODY	93.27	H
MEÇ17009.01	115.60	C
MEÇ17009.02	97.57	GH
MEÇ17011.01	98.10	GH
MEÇ17011.03	80.94	JKL
MEÇ17011.11	108.50	DE
MEÇ17011.13	79.53	JKLM
MEÇ17011.15	138.10	A
MEÇ17011.17	77.98	KLMN
MEÇ17011.18	94.51	H
MEÇ17013.03	76.72	LMN
MEÇ17023.01	63.99	O
MEÇ17023.02	83.22	IJ
MEÇ17023.03	93.42	H
MEÇ17023.04	76.70	LMN
MEÇ17024.02	128.90	B
MEÇ17053.04	86.02	I
MEÇ17065.02	75.59	MN
MEÇ17075.01	136.90	A
MEÇ17075.08	113.50	C
MEÇ17076.02	104.20	EF
MEÇ17076.03	83.23	IJ
MEÇ17078.03	112.90	CD
MEÇ17078.06	63.67	O
MEÇ17080.01	75.30	MN
MEÇ17080.06	82.25	IJK
MEÇ17080.14	94.51	H
MEÇ17084.01	65.64	O
MEÇ17092.03	73.66	N
Mean value	91.64	

*Different letters within column indicate significance at p≤0.05 LSD test

4.5 Tuber size grading (%)

4.5.1 First Class Tuber Ratio (%)

The ANOVA of the first-class tuber (> 50mm) ratio revealed that there were highly significant ($P<0.001$) differences among the 33 genotypes with a coefficient of variation (CV) of 10.75% (Table 4.9). In Table 4.10 the average rate of the first-class tuber was found to be 68.45% with the genotypic ratios ranging from 61.1% to 81.99% which belonged to MEÇ17065.02 and MEÇ17009.01 respectively. This range of differences in tuber number indicates that some of the genotypes are high yielding whiles others are medium yielding and others low yielding, explainable due to different pedigree sources of these genotypes. However, the non-significant difference among the replication is an indication that each of the genotypes are behaviourally stable across the environmental field.

These findings confirm the results of Pandey et al. (2017), and Naawe (2020), but contradict the findings of Islam Pulok et al. (2016). Naawe (2020) found significant differences among tuber grading of potato breeding lines in three different locations. Which indicated that the proportion tuber grading of the breeding lines is was affected by genotype. On the contrarily, Pulok et al. (2016), reported that there were no significant differences in the first-class tuber ratio of the potato cultivars across location and replications.

Table 4.9. Analysis of variance of first-class tuber (> 50mm) ratio of potato genotypes grown in Nigde

Source	DF	SS	MS	FV	P-value
Replication	3	71.92	23.697	0.4374	0.0000**
Genotype	32	8139.080	254.346	4.6944	0.0000**
Error	96	5201.385	54.181		
Total	131	113411.557			
CV%	10.75				

DF= degree of freedom, SS= sum of square, MS= mean square, CV= coefficient of variation FV= F value, **= high significant,

Table 4.10. Average values of first class tuber (> 50mm) ratio of potato genotypes grow in Nigde

Treatment	Big Tuber (> 50mm) Yield	Group Letter*
AGRIA	71.92	ABCDEFG
ALEGRIA	76.03	ABCD
L.OLYMPIA	72.65	ABCDEFG
MARABEL	62.72	GHIJK
MELODY	78.22	ABC
MEÇ17009.01	81.99	A
MEÇ17009.02	74.46	ABCDE
MEÇ17011.01	78.17	ABC
MEÇ17011.03	70.91	BCDEFGHI
MEÇ17011.11	71.77	ABCDEFGH
MEÇ17011.13	63.00	GHIJK
MEÇ17011.15	79.99	AB
MEÇ17011.17	67.41	DEFGHIJ
MEÇ17011.18	65.27	EFGHIJK
MEÇ17013.03	55.87	KL
MEÇ17023.01	61.5	HIJK
MEÇ17023.02	64.19	EFGHIJK
MEÇ17023.03	72.03	ABCDEFG
MEÇ17023.04	63.63	FGHIJK
MEÇ17024.02	68.03	CDEFGHI
MEÇ17053.04	65.36	EFGHIJK
MEÇ17065.02	61.10	IJK
MEÇ17075.01	71.76	ABCDEFGH
MEÇ17075.08	79.01	AB
MEÇ17076.02	77.43	ABCD
MEÇ17076.03	68.54	CDEFGHIJ
MEÇ17078.03	73.44	ABCDEF
MEÇ17078.06	71.92	L
MEÇ17078.03	76.03	JK
MEÇ17080.06	72.65	EFGHIJK
MEÇ17080.14	62.72	BCDEFGHI
MEÇ17084.01	78.22	EFGHIJK
MEÇ17092.03	81.99	KL
Mean value	68.45	

*Different letters within column indicate significance at p≤0.05 LSD test

4.5.2 Second Class Tuber Ratio (%)

The ANOVA of the second-class tuber (30-50 mm) ratio showed there were highly significant ($p < 0.001$) effects of genotypes, with a CV of 15.48 in (Table 4.11). The second-class mean tuber number of the genotypes ranged from 15.83% to 48.19% occurring at MEÇ17009.01 and MEÇ17078.06 respectively with an average of 27.19%. This difference can be attributed to the different genotypic origins of the genotypes. On the other hand, the non-significant differences among the replication suggest that replication does not affect the second-class tuber rate of the genotypes; (Table 4.12).

Table 4.11. Analysis of variance of second class tuber (30-50 mm) ratio of 28 potato breeding lines and five cultivars grow in Nigde

Source	DF	SS	MS	FV	P-value
Replication	3	42.259	14.086	0.2936	0.0000**
Genotype	32	5411.917	168.122	3.5244	0.0000**
Error	96	4606.634	5.940		
Total	131	10060.810			
CV%	15.48				

DF= degree of freedom, SS= sum of square, MS= mean square, CV= coefficient of variation FV= F value, **= high significant

Table 4.12. Average values of second class tuber (30-50 mm) ratio of potato genotypesgrown in Nigde

Treatment	second class tuber (30-50 mm) ratio	Group Letter*
AGRIA	24.68	DEFGHIJK
ALEGRIA	21.43	FGHIJK
L.OLYMPIA	25.74	DEFGHIJ
MARABEL	31.65	BCDE
MELODY	19.11	HIJK
MEÇ17009.01	15.83	K
MEÇ17009.02	23.78	EFGHIJK
MEÇ17011.01	19.36	HIJK
MEÇ17011.03	26.82	DEFGHIJ
MEÇ17011.11	25.21	DEFGHIJK
MEÇ17011.13	33.79	BCD
MEÇ17011.15	17.12	JK
MEÇ17011.17	28.68	BCDEFGH
MEÇ17011.18	31.59	BCDE
MEÇ17013.03	37.5	B
MEÇ17023.01	31.09	BCDEF
MEÇ17023.02	27.44	CDEFGHI
MEÇ17023.03	23.52	EFGHIJ
MEÇ17023.04	30.29	BCDEFG
MEÇ17024.02	27.16	CDEFGHI
MEÇ17053.04	28.78	BCDEFGH
MEÇ17065.02	27.39	CDEFGHI
MEÇ17075.01	24.49	DEFGHIJK
MEÇ17075.08	18.72	IJK
MEÇ17076.02	20.71	GHIJK
MEÇ17076.03	28.04	BCDEFGHI
MEÇ17078.03	24.17	DEFGHIJK
MEÇ17078.06	48.19	A
MEÇ17078.03	32.94	BCDE
MEÇ17080.06	30.34	BCDEFG
MEÇ17080.14	26.63	DEFGHIJ
MEÇ17084.01	28.25	BCDEFGHIJ
MEÇ17092.03	36.68	BC
Mean value	27.19	

*Different letters within column indicate significance at p≤0.05 LSD test

4.5.3 Third Class Tuber Ratio (%)

The ANOVA for Third Class Tuber (< 30mm) ratio revealed a highly significant ($p < 0.001$) effect of genotypes on the potato tuber rates (Table 4.13, Table 4.14). The number of the Third-Class Tuber (< 30mm) Ratio ranges from Landy Olympia (2.54%) to MEÇ17065.02 (11.50%) with the average being 4.36%.

Table 4.13. Analysis of variance of third class tuber (< 30mm) ratio of potato genotypes grow in Nigde

Source	DF	SS	MS	FV	P-value
Replication	3	4.879	1.626	0.8183	0.0000**
Genotype	32	731.507	22.292	11.2187	0.0000**
Error	96	190.799	1.987		
Total	131	909.185			
CV%	12.28				

DF= degree of freedom, SS= sum of square, MS= mean square, CV= coefficient of variation FV= F value, **= high significant

Table 4.14. Average values of third class tuber (< 30mm) ratio of potato genotypes grown in Nigde

Treatment	Small tuber (< 30mm) yield	Group Letter*
AGRIA	3.40	HIJKL
ALEGRIA	2.54	IJKL
L.OLYMPIA	1.61	L
MARABEL	5.64	CDEFG
MELODY	2.67	IJKL
MEÇ17009.01	2.17	JKL
MEÇ17009.02	1.75	L
MEÇ17011.01	2.47	JKL
MEÇ17011.03	2.26	JKL
MEÇ17011.11	3.02	HIJKL
MEÇ17011.13	3.20	HIJKL
MEÇ17011.15	2.89	HIJKL
MEÇ17011.17	3.91	FGHIJ
MEÇ17011.18	3.14	HIJKL
MEÇ17013.03	6.62	BCD
MEÇ17023.01	7.41	BC
MEÇ17023.02	8.37	B
MEÇ17023.03	4.46	EFGHI
MEÇ17023.04	6.07	CDE
MEÇ17024.02	4.80	DEFGH
MEÇ17053.04	5.86	CDEF
MEÇ17065.02	11.50	A
MEÇ17075.01	3.74	GHIJK
MEÇ17075.08	2.27	JKL
MEÇ17076.02	1.86	KL
MEÇ17076.03	3.42	HIJKL
MEÇ17078.03	2.38	JKL
MEÇ17078.06	5.59	CDEFG
MEÇ17078.03	7.54	BC
MEÇ17080.06	4.71	DEFGH
MEÇ17080.14	2.25	JKL
MEÇ17084.01	7.28	BC
MEÇ17092.03	7.22	BC
Mean value	4.36	

*Different letters within column indicate significance at p≤0.05 LSD test

4.6 Total tuber yield t/ha

The analysis variance of the total tuber yield showed highly significant differences among the genotypes occurring at a CV of 3.38 % (Table 4.15) and an average tuber yield of 30.83 tons/ha. The maximum total tuber yield/ha was recorded in MEÇ17011.11 (48.23t/ha) whereas the lowest total tuber yield was recorded in MEÇ17023.01 (15.15t/ha). This wide range in the tuber yield of the genotypes shows that potato genotypes have a different genetic background and yielding capacity. This is in line with the finding of Arioglu et al., (2010) that tuber yields are affected by the genotypes difference.

Table 4.15. Analysis of variance of total tuber yield/ha of potato genotypes grown in Nigde

Source	DF	SS	MS	FV	P-value
Replication	3	5.129	1.710	1.5744	0.2006ns
Genotype	32	11671.402	364.731	335.8627	0.0000**
Error	96	104.252	1.086		
Total	131	11780.783			
CV%	3.38				

DF= degree of freedom, SS= sum of square, MS= mean square, CV= coefficient of variation FV= F value, **= high significant, ns= no significant

Table 4.16. Average values of total tuber yield/ha of potato genotypes grown in Nigde

Treatment	Total tuber yield (t/ha)	Group Letter*
AGRIA	33.32	G
ALEGRIA	32.90	G
L.OLYMPIA	36.35	EF
MARABEL	15.61	QR
MELODY	32.98	G
MEÇ17009.01	33.29	G
MEÇ17009.02	17.40	OP
MEÇ17011.01	34.00	G
MEÇ17011.03	40.38	D
MEÇ17011.11	48.23	A
MEÇ17011.13	27.52	HI
MEÇ17011.15	44.20	B
MEÇ17011.17	37.27	E
MEÇ17011.18	42.26	C
MEÇ17013.03	24.64	KL
MEÇ17023.01	15.15	R
MEÇ17023.02	19.63	N
MEÇ17023.03	27.94	H
MEÇ17023.04	23.99	L
MEÇ17024.02	37.66	E
MEÇ17053.04	26.14	IJ
MEÇ17065.02	16.56	PQR
MEÇ17075.01	41.54	CD
MEÇ17075.08	40.60	D
MEÇ17076.02	37.80	E
MEÇ17076.03	25.74	JK
MEÇ17078.03	35.72	F
MEÇ17078.06	44.76	B
MEÇ17080.01	21.45	M
MEÇ17080.06	33.89	G
MEÇ17080.14	33.21	G
MEÇ17084.01	41.54	CD
MEÇ17092.03	40.60	D
Mean value	30.83	

*Different letters within column indicate significance at $p \leq 0.05$ LSD test

4.7 Marketable Tuber Yield (t/ha)

The analysis variance of the marketable tuber yield showed a highly significant ($P< 0.001$) difference among the with a CV of 3.77 % (Table 4.17) and the average tuber yield of 29.63 tons/ha. The maximum marketable tuber yield/ha was recorded in MEÇ17011.11 (46.52 tons/ha) whereas the lowest marketable tuber yield was recorded in MEÇ17023.01 (13.01 tons/ha). This wide range in the marketable tuber yield of the genotypes shows that the genotypes have greater effects on the yield outcome depicting their diverse pedigree sources. Habtamu et al. (2016) found similar results for the marketable tuber yield.

Table 4.17. Analysis of variance of marketable tuber yield/ha of potato genotypes grown in Nigde

Source	DF	SS	MS	FV	P-value
Replication	3	6.982	2.327	1.8683	0.1401ns
Genotype	32	11671.228	364.726	292.7830	0.0000**
Error	96	119.589	1.246		
Total	131	11797.799			
CV%	3.77				

DF= degree of freedom, SS= sum of square, MS= mean square, CV= coefficient of variation FV= F value, **= high significant, ns= no significant

Table 4.18. Average values of marketable tuber yield/ha of potato genotypes grown in Nigde

Treatment	Marketable tuber yield/ha (t/ha)	Group Letter
AGRIA	32.19	G
ALEGRIA	32.06	G
L.OLYMPIA	35.76	EF
MARABEL	14.73	OP
MELODY	32.10	G
MEÇ17009.01	32.63	G
MEÇ17009.02	17.02	MN
MEÇ17011.01	33.29	G
MEÇ17011.03	39.46	D
MEÇ17011.11	46.52	A
MEÇ17011.13	26.76	H
MEÇ17011.15	42.86	B
MEÇ17011.17	35.80	EF
MEÇ17011.18	40.87	CD
MEÇ17013.03	22.84	JK
MEÇ17023.01	13.93	P
MEÇ17023.02	18.08	M
MEÇ17023.03	26.94	H
MEÇ17023.04	22.31	K
MEÇ17024.02	36.15	EF
MEÇ17053.04	24.29	IJ
MEÇ17065.02	14.60	OP
MEÇ17075.01	40.83	CD
MEÇ17075.08	39.68	D
MEÇ17076.02	37.22	E
MEÇ17076.03	24.82	I
MEÇ17078.03	34.88	F
MEÇ17078.06	41.84	BC
MEÇ17080.01	19.78	L
MEÇ17080.06	32.28	G
MEÇ17080.14	32.40	G
MEÇ17084.01	15.71	NO
MEÇ17092.03	17.15	MN
Mean value	29.63	

*Different letters within column indicate significance at p≤0.05 LSD test

4.8 Specific gravity (SG)

It was revealed by the ANOVA (Table 4.19) that, high significant ($p < 0.001$) effects occur among the genotypes and replication for the specific gravity (SG) with a CV 0.77. The maximum specific gravity of 1.103 was found in AGRIA, MEÇ17065.02, and ALEGRIA while the lowest specific gravity of 1.069 was found in MEÇ17023.03, MELODY, and MEÇ17076.03. The significant genotypic effect on the SG on the genotypes indicates that they vary in their genetic constitution which greatly influenced the quality traits. This result confirms the findings of (Tessema *et al*. 2020) that SG is influenced by genetic varietal differences. In the potato production and food processing industry, SG is an important parameter especially for French fries or chips (Kabira and Berg, 2003; CIP, 2007; Wassu, 2016). For French fry and potato chips, (CIP (2007) set the minimum acceptable levels of potato tubers with a specific gravity of ≥1.080, which reduces the amount of oil uptake by potato chips and French fries during frying and enhanced texture and yield quality of finished products. This indicates that breeding lines with their SG less than 1.080 are not suitable for food processing

Table 4.19. Analysis of variance of specific gravity of potato genotypes grown in Nigde

Source	DF	SS	MS	FV	P-value
Replication	3	0.00011	3.688		0.0000**
Genotype	32	0.00422	1.317	1.86	0.0000**
Error	96	0.00681	7.089		
Total	131	0.01113			
CV%	0.77				

DF= degree of freedom, SS= sum of square, MS= mean square, CV= coefficient of variation FV= F value, **= high significant

Table 4.20. Average values of specific gravity of potato genotypes grown in Nigde

Treatment	Specific gravity (SG)	Group Letter*
AGRIA	1.103	A
ALEGRIA	1.098	AB
L.OLYMPIA	1.095	ABC
MARABEL	1.094	ABC
MELODY	1.098	D
MEÇ17009.01	1.093	ABC
MEÇ17009.02	1.089	BC
MEÇ17011.01	1.088	BC
MEÇ17011.03	1.092	ABC
MEÇ17011.11	1.095	ABC
MEÇ17011.13	1.091	ABC
MEÇ17011.15	1.092	ABC
MEÇ17011.17	1.091	ABC
MEÇ17011.18	1.084	C
MEÇ17013.03	1.094	ABC
MEÇ17023.01	1.096	ABC
MEÇ17023.02	1.092	ABC
MEÇ17023.03	1.069	D
MEÇ17023.04	1.096	ABC
MEÇ17024.02	1.088	BC
MEÇ17053.04	1.096	ABC
MEÇ17065.02	1.098	AB
MEÇ17075.01	1.088	BC
MEÇ17075.08	1.092	ABC
MEÇ17076.02	1.092	ABC
MEÇ17076.03	1.087	BC
MEÇ17078.03	1.097	AB
MEÇ17078.06	1.090	BC
MEÇ17084.01	1.087	BC
MEÇ17080.06	1.088	BC
MEÇ17080.14	1.089	BC
MEÇ17084.01	1.088	BC
MEÇ17092.03	1.090	BC
Mean value	1.092	

*Different letters within column indicate significance at p≤0.05 LSD test

4.9 Dry matter content (%)

The ANOVA revealed highly significant ($p < 0.001$) effects of the genotypes, on the dry matter content (DMC) (Table 4.21) with a CV of 2.14. The DMC of the genotypes ranged from 17.29% to 23.26 % with the highest % DMC observed in MEÇ17011.11 (23.26) whereas the lowest % DMC was observed in MEÇ17023.01 (17.29) (Table 4.22). This indicates that the difference in the DMC among the genotypes is largely due to their differential pedigree origins and quality traits behaviors. These findings confirm the results Celik et al. (2006) and Arioglu et al. (2016).

Table 4.21. Analysis of variance of dry matter content of potato genotypes grown in Nigde

Source	DF	SS	MS	FV	P-value
Replication	3	1.445	0.482	2.5289	0.0618ns
Genotype	32	259.741	8.117	42.6154	0.0000**
Error	96	18.285	0.190		
Total	131	279.471			
CV%	2.14				

DF= degree of freedom, SS= sum of square, MS= mean square, CV= coefficient of variation FV= F value, **= high significant, ns= no significant

Table 4.22. Average values of dry matter content of potato genotypes grown in Nigde

Treatment	Dry Matter Content (DMC)	Group Letter*
AGRIA	20.48	GHI
ALEGRIA	19.91	IJK
L.OLYMPIA	20.36	HIJ
MARABEL	17.29	N
MELODY	17.98	M
MEÇ17009.01	21.51	D
MEÇ17009.02	20.29	HIJ
MEÇ17011.01	20.32	HIJ
MEÇ17011.03	23.15	AB
MEÇ17011.11	23.26	A
MEÇ17011.13	20.33	HIJ
MEÇ17011.15	22.59	BC
MEÇ17011.17	22.44	C
MEÇ17011.18	21.08	DEFG
MEÇ17013.03	20.78	EFGH
MEÇ17023.01	19.35	KL
MEÇ17023.02	19.56	KL
MEÇ17023.03	17.83	MN
MEÇ17023.04	18.28	M
MEÇ17024.02	19.16	L
MEÇ17053.04	19.46	KL
MEÇ17065.02	19.80	JK
MEÇ17075.01	20.64	FGH
MEÇ17075.08	20.65	FGH
MEÇ17076.02	20.44	HI
MEÇ17076.03	20.51	GHI
MEÇ17078.03	20.19	HIJ
MEÇ17078.06	21.28	DE
MEÇ17080.01	21.44	D
MEÇ17080.06	21.59	D
MEÇ17080.14	21.15	DEF
MEÇ17084.01	21.57	D
MEÇ17092.03	19.14	L
Mean value	20.42	

*Different letters within column indicate significance at p≤0.05 LSD test

4.10 **Screening of Potato Genotypes with Molecular Markers**

Potato virus Y and potato virus X causing significant yield losses in potato cultivation are socio-economic important biotic stress factors and it is good to get developed varieties that have desirable characteristics and genetic resistance because varieties that are of less resistance to viral agents are economically weak for yield maximization. All the genotypes in this experiment were screened for resistance against PVY and PVX. This was done by collecting and evaluating all the genotypes. Young leaf samples were collected during the flowering period and used for genomic DNA extraction. Agarose gel images of genomic DNA samples obtained after isolation are given in Figure 4.1

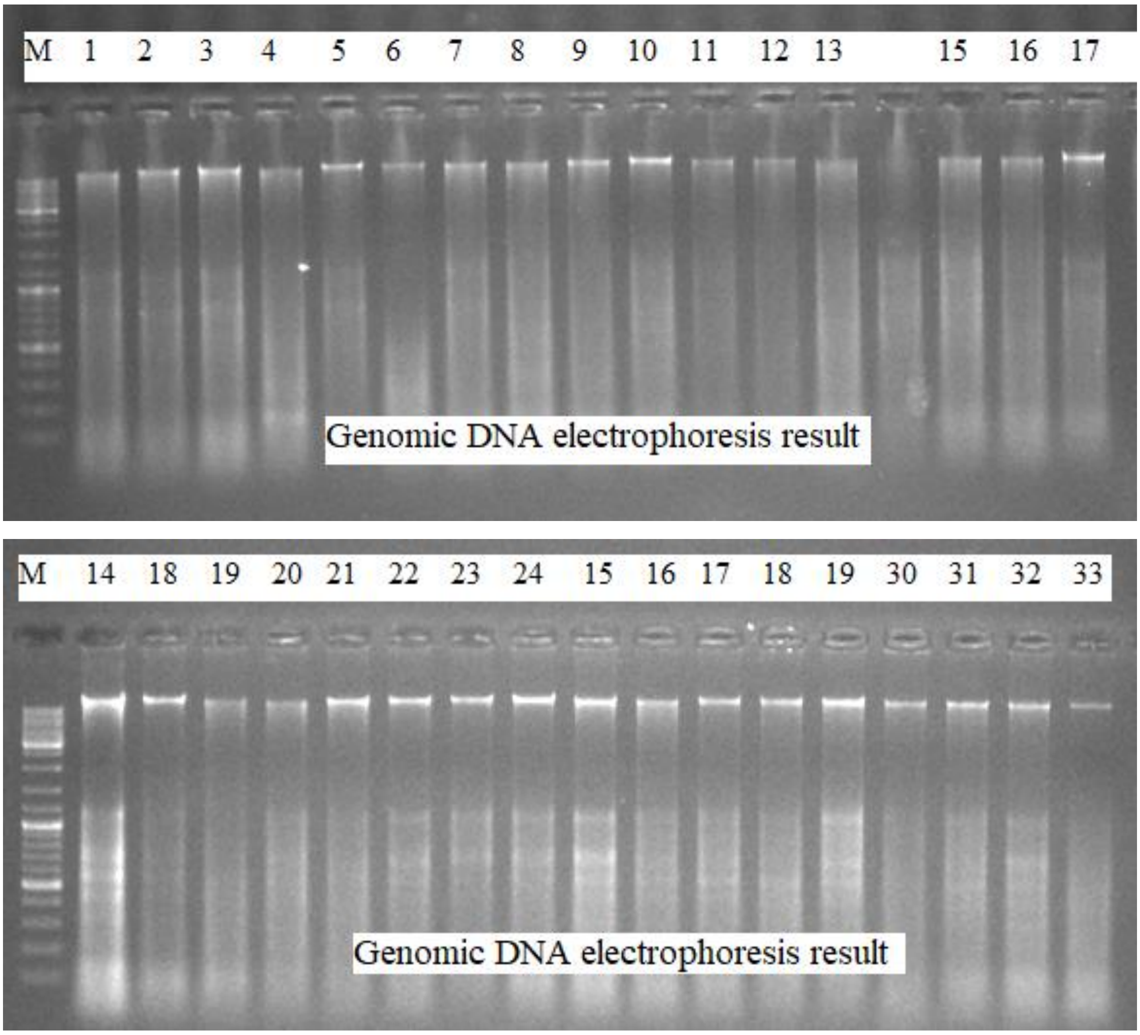

Figure 4.1. Agarose gel electrophoresis of isolated genomic DNA of 33 potato genotypes 1=AGRIA,2=ALEGRIA,3=L.OLYMPIA,4=MARABEL,5=MELODY,6=MEÇ17009.01,7=MEÇ17009.02,8=MEÇ17011.01,9=MEÇ17011.03,10=MEÇ17011.11,11=MEÇ17011.13,12=MEÇ17011.15,13=MEÇ17011.17,14=MEÇ17011.18,15=MEÇ17013.03,16=MEÇ17023.01,17=MEÇ17023.02,18=MEÇ17023.03,19=MEÇ17023.04,20=MEÇ17024.02,21=MEÇ17053.04,22=MEÇ17065.02,23=MEÇ17075.01,24=MEÇ17075.08,25=MEÇ17076.02,26=MEÇ17076.03,27=MEÇ17078.03,28=MEÇ17078.06,29=MEÇ17080.01,30=MEÇ17080.06,31=MEÇ17080.14,32=MEÇ17084.01,33=MEÇ17092.03

4.11 PVX PCR Product Result

The extracted genomic DNA samples from the potato genotypes were screened for resistance to PVX. To prove resistance in the genotypes investigated, a couple of primers were used; 1Rx1 and 106Rx2. If a genotype sample has one of two genes, it was considered resistant to PVX. So, the two genes are different for their amplicon length. Rx2 gene with 543 bp and Rx1 gene with 974 bp were amplified, which informed resistance (Ahmadvand R. et al., 2013). Rx1 was amplified by primers 1Rx1, while Rx2 was amplified by primers 106Rx2. Figure 4.2 shows the PCR result for PVX resistance of the genotypes.

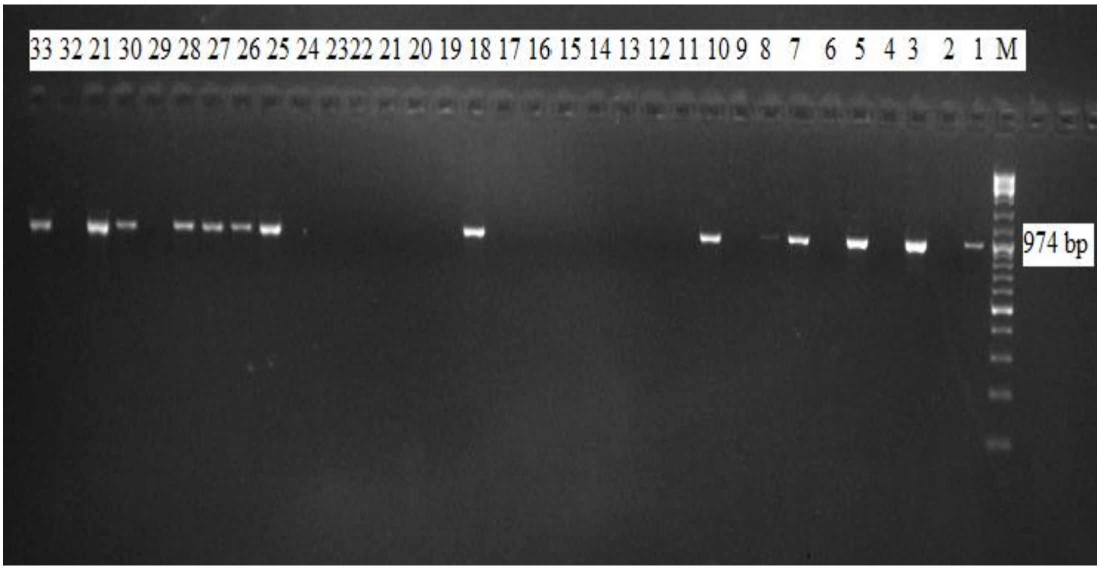

Figure 4.2. PCR results of RX1 gene 1=AGRIA,2=ALEGRIA,3=L.OLYMPIA,4=MARABEL,5=MELODY,6=MEÇ17009.01,7=MEÇ17009.02,8=MEÇ17011.01,9=MEÇ17011.03,10=MEÇ17011.11,11=MEÇ17011.13,12=MEÇ17011.15,13=MEÇ17011.17,14=MEÇ17011.18,15=MEÇ17013.03,16=MEÇ17023.01,17=MEÇ17023.02,18=MEÇ17023.03,19=MEÇ17023.04,20=MEÇ17024.02,21=MEÇ17053.04,22=MEÇ17065.02,23=MEÇ17075.01,24=MEÇ17075.08,25=MEÇ17076.02,26=MEÇ17076.03,27=MEÇ17078.03,28=MEÇ17078.06,29=MEÇ17080.01,30=MEÇ17080.06,31=MEÇ17080.14,32=MEÇ17084.01,33=MEÇ17092.03. The sample that has a 974 bp band is resistant

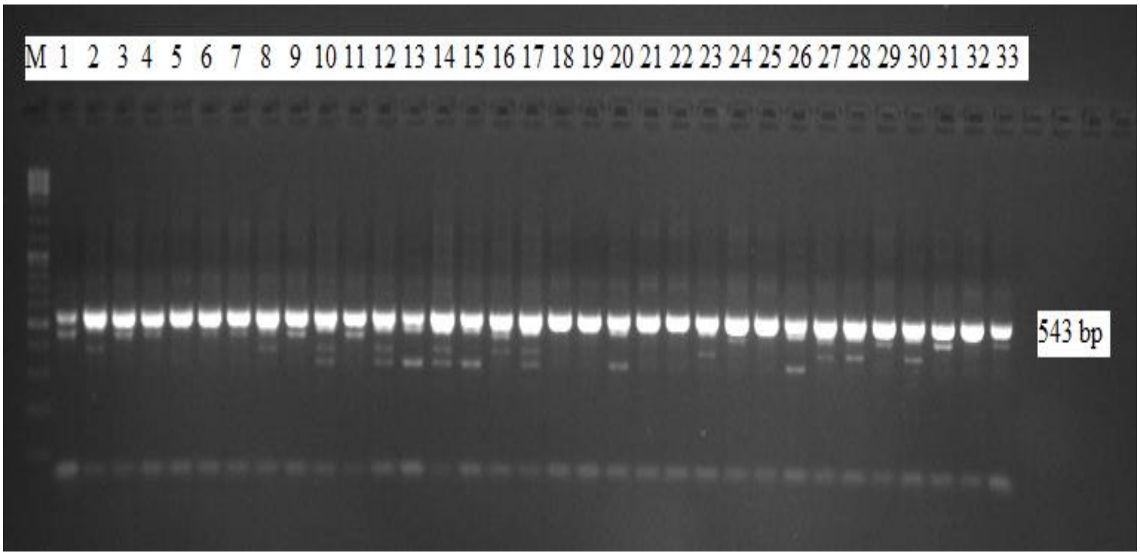

Figure 4.3. PCR results of RX2 gene 1=AGRIA,2=ALEGRIA,3=L.OLYMPIA,4=MARABEL,5=MELODY,6=MEÇ17009.01,7=MEÇ17009.02,8=MEÇ17011.01,9=MEÇ17011.03,10=MEÇ17011.11,11=MEÇ17011.13,12=MEÇ17011.15,13=MEÇ17011.17,14=MEÇ17011.18,15=MEÇ17013.03,16=MEÇ17023.01,17=MEÇ17023.02,18=MEÇ17023.03,19=MEÇ17023.04,20=MEÇ17024.02,21=MEÇ17053.04,22=MEÇ17065.02,23=MEÇ17075.01,24=MEÇ17075.08,25=MEÇ17076.02,26=MEÇ17076.03,27=MEÇ17078.03,28=MEÇ17078.06,29=MEÇ17080.01,30=MEÇ17080.06,31=MEÇ17080.14,32=MEÇ17084.01,33=MEÇ17092.03The sample that has a 543bp band is resistant

4.12 PVY PCR Product Result

The PCR screening of the extracted genomic DNA samples shows resistance of the genotypes to PVY. Thus, STM0003 primer was used to diagnose absence and presence of Ry_{sto} respectively. PVYsto is represented by two bands and amplicon sizes of the bands are 111-140 bp (Song, Y.S. et al., 2005).

Rysto provide resistance against PVY if two bands exist. 141 bp bands observe every time but it is not enough to provide resistance. Therefore 111 bp bands are necessary to be able to defeat PVY (Song, Y.S. et al., 2005). So, genotypes which have two bands are accepted as PVY resistant. The result PVY scanning is represented in Figure 4.4.

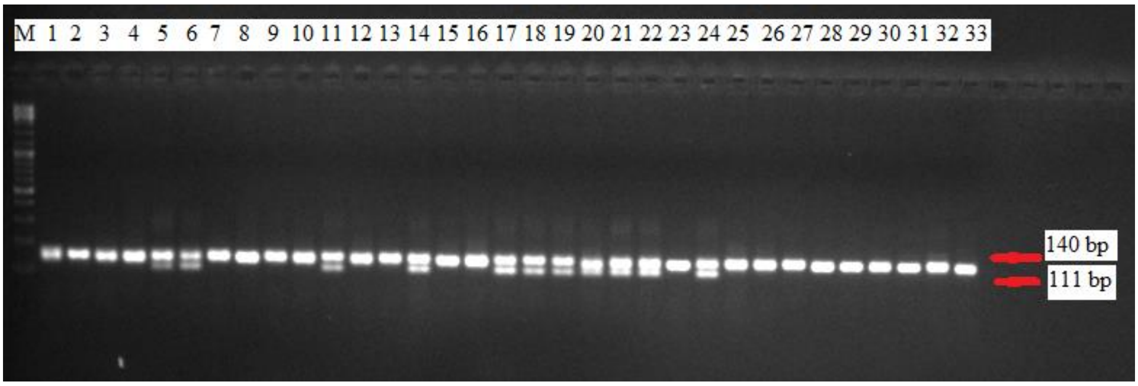

Figure 4.4 PCR results of RXTM003 gene 1=AGRIA,2=ALEGRIA,3=L.OLYMPIA,4=MARABEL,5=MELODY,6=MEÇ17009.01,7=MEÇ17009.02,8=MEÇ17011.01,9=MEÇ17011.03,10=MEÇ17011.11,11=MEÇ17011.13,12=MEÇ17011.15,13=MEÇ17011.17,14=MEÇ17011.18,15=MEÇ17013.03,16=MEÇ17023.01,17=MEÇ17023.02,18=MEÇ17023.03,19=MEÇ17023.04,20=MEÇ17024.02,21=MEÇ17053.04,22=MEÇ17065.02,23=MEÇ17075.01,24=MEÇ17075.08,25=MEÇ17076.02,26=MEÇ17076.03,27=MEÇ17078.03,28=MEÇ17078.06,29=MEÇ17080.01,30=MEÇ17080.06,31=MEÇ17080.14,32=MEÇ17084.01,33=MEÇ17092.03. The sample that has 111-140bp is resistant to PVY

To PVX resistance just one band is enough. So, susceptibility or resistance was listed (Table 4.23) in terms of one band for PVX resistance. In addition, PVY resistance is also examined in (Table 4.23)

Table 4.23. Resistance and susceptibility of genotypes against PVY and PVX at a glance

		PVX			PVY	
NO	Samples	1Rx1	106Rx2	OVERAL	STM0003	OVERAL
1	17009.01	+	+	R	-	S
2	17009.02	-	+	R	-	S
3	17011.01	+	+	R	-	S
4	17011.03	-	+	R	-	S
5	17011.11	+	+	R	+	R
6	17011.13	-	+	R	+	R
7	17011.15	+	+	R	-	S
8	17011.17	+	+	R	-	S
9	17011.18	-	+	R	-	S
10	17013.03	+	+	R	-	S
11	17023.01	-	+	R	+	R
12	17023.02	-	+	R	-	S
13	17023.03	-	+	R	-	S
14	17023.04	-	+	R	+	R
15	17024.02	-	+	R	-	S
16	17053.04	-	+	R	-	S
17	17065.02	-	+	R	+	R
18	17075.01	+	+	R	+	R
19	17075.08	-	+	R	+	R
20	17076.02	-	+	R	+	R
21	17076.03	-	+	R	+	R
22	17078.03	-	+	R	+	R
23	17078.06	-	+	R	-	S
24	17080.01	-	+	R	+	R
25	17080.06	+	+	R	-	S
26	17080.14	+	+	R	-	S
27	17084.01	+	+	R	-	S
28	17092.03	+	+	R	-	S
29	Agria	-	+	R	-	S
30	Alegria	+	+	R	-	S
31	L.Olympia	+	+	R	-	S
32	Marabel	-	+	R	-	S
33	Melody	+	+	R	-	S

+, resistant(R) against virus strain; - susceptible(S) against virus strain

The resistant genes which are Ry_{sto} for PVY resistance, Rx1 and Rx2 for PVX resistance were screened with the molecular selection markers. As a result of the study, all 33 genotypes have resistance genes against PVX, and 11 out of 33 genotypes have Ry_{sto} gene regulating resistance against PVY. Thus MEÇ17011.11, MEÇ17011.13, MEÇ17023.01, MEÇ17023.04, MEÇ17065.02, MEÇ17075.01, MEÇ17075.08, MEÇ17076.02, MEÇ17076.03, MEÇ17078.03, MEÇ17080.01 genotypes were proven to have resistance genes against both PVY and PVX.

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CHAPTER V

CONCLUSION

In potato cultivation and breeding programs, abiotic and biotic stresses are important factors to consider. Among the biotic factors, virus infestation of potato causes severe yield losses and total crop failure. It is thus paramount to consider virus screening in potato breeding programs to develop potato varieties that are high yielding and resistant to viruses such as potato virus X (PVX) and the potato virus Y (PVY). This study was hence designed in that regards to screen potato breeding lines that are high yielding and resistant to the PVX and PVY strains. The key findings of this study are hence below.

- There were high genotypic variabilities ($p \leq 0.01$) among the genotypes for all the traits accessed.
- Out of the 28 breeding lines, 8 of them (MEÇ17011.11, MEÇ17078.06, MEÇ17011.15, MEÇ17011.18, MEÇ17075.01, MEÇ17084.01, MEÇ17075.08, and MEÇ17092.03) have total tuber yield ≥ 40 t/ha and are hence considered as high yielding breeding lines.
- For the virus screening, all breeding lines, and standard cultivars showed resistant against PVX strain while 11 genotypes are resistant against PVY strain.
- Genotypes MEÇ17011.11, MEÇ17011.13, MEÇ17023.01, MEÇ17023.04, MEÇ17065.02, MEÇ17075.01, MEÇ17075.08, MEÇ17076.02, MEÇ17076.03, MEÇ17078.03, and MEÇ17080.01 are resistant against both PVY and PVX strains.
- The genotypes MEÇ17011.11, MEÇ17075.08, MEÇ17011.18, and MEÇ17075.01 are high yielding and resistant to both PVX and PVY virus strains and hence can be considered as candidate potato breeding lines.
- It is suggested that further evaluation of promising breeding lines for yield, tuber quality and resistance traits under different environmental conditions to decide the cultivar candidates for release.

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