GENETIC DIVERSITY OF POTATO ACCESSIONS REVEALED BY SIMPLE SEQUENCE REPEAT MARKERS

By

Tasnim Khandakerr Samiha
21276008

A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
Masters in Science

Mathematics and Natural Sciences
Brac University
September 2023

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.
5. I would like to request the embargo of my thesis for 24M from the submission date

Student's Full Name & Signature:

_____ Tasnim Khandker Samiha_____
Student Full Name
Student ID : 21276008

Approval

The thesis/project titled “Genetic diversity of potato accessions revealed by Simple Sequence Repeat markers” submitted by”

1. Tasnim Khandaker Samiha (21276008)

{of Fall, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Masters of Science in Biotechnology on [28-12-2023].}

Examining Committee:

Supervisor: (Member)	Dr. Aparna Islam MS and BS Biotechnology Program Professor, Department of Mathematics and Natural Sciences, BRAC University
	_____
Co-Supervisor: (Member)	Dr. Abu Shamim Mohammad Nahiyan, Principal Scientist at ASRBC (Advanced Seed Research and Biotech Center), ACI Limited, Bangladesh
	_____
Program Director: (Member)	Dr. Munima Haque MS and BS Biotechnology Director & Associate Professor Department of Mathematics and Natural Sciences, BRAC University
	_____
External Expert Examiner: (Member)	Dr. Rakha Hari Sarker_ Professor Emeritus, Department of Botany, University of Dhaka
	_____
Departmental Head: (Chair)	Dr. A F M Yusuf Haider Professor and Chairperson Department of Mathematics and Natural Sciences, BRAC University
	_____

Ethics Statement

There is no conflict of interest.

Abstract

Potato is one of the most economically important food crops worldwide. Information on the genetic diversity of a germplasm bank is necessary for potato breeding. In this study, genetic diversity of 62 potato accessions developed by ACI, Limited, Bangladesh, have been analyzed using 9 simple sequence repeat (SSR) markers. We identified 46 unique alleles for the 9 loci, with 93.5% showing polymorphism. The number of alleles ranged from three to eleven with the average being 7.3. The polymorphic information content per locus ranged from 0.621 to 0.888, with an average of 0.787. The accessions showed significant diversity since the calculated Jaccard's similarity coefficient ranged from 0.182 to 0.778. Analysis using the unweighted pair-group method arithmetic average (UPGMA) grouped the accessions in 5 clusters. Evaluation of the SSR primers will help in future genetic diversity studies, while the diversity information will aid in parental selection in breeding programs.

Keywords: genetic diversity; gene bank; microsatellite; polymorphism; SSR markers; Solanum tuberosum L.

Acknowledgement

We thank Dr. Fareyzul Haque Ansarey, President of ACI Agribusinesses, ACI Ltd., Professor Dr. Md. Abdus Siddique, Former Advisor of ASRBC, ACI Ltd., and Former Professor of Bangladesh Agricultural University, as well as Professor Dr. Lutfur Rahman, Former Chief Advisor of ASRBC, ACI Ltd., and Former Professor of Bangladesh Agricultural University for their unwavering support on this research project.

Table of Contents

List of Tables

List of Figures

List of Acronyms

FAO	Food and Organization of the United Nations
PIC	Polymorphic Information Content
RDCC	Rate of Distinguishing Cultivars by Clusters
SSR	Simple Sequence Repeats
UPGMA	unweighted pair-group method with arithmetic average

Chapter 1

[Introduction]

Prevalence of Potato Production

Potato (*Solanum Tuberosum L.*), which is a part of the Solanaceae family, is one of the most important food crops worldwide (Uddin et al., 2015). Globally, its ranking is the third most consumed food crop, after rice and wheat (Devaux et al., 2014). Moreover, potato is the single most important non-cereal crop worldwide, making it a crucial crop in the global food system (Roy, 2017).

Potato is considered, relative to other major crops, significantly higher yielding since it produces more food in less time per unit of cropland (Devaux et al., 2014). In addition, potatoes are nutritionally-dense in terms bioavailability and bioaccessibility of minerals and vitamins to human health (Devaux et al 2021; Burgos et al., 2020). Its high nutritional value also gives potato the capability to improve the micronutrient deficiency, also called “hidden hunger”, affecting people especially in the developing countries (Devaux et al., 2021).

Despite the improvement in food security worldwide, feeding the expanding population remains a challenge (Devaux et al., 2021). With numerous other challenges at the current global state such as various conflicts, inequality, population growth and the COVID-19 pandemic, and also future unexpected challenges such as the climate change; potato production provides promising potential to resolve both food security and income generation.

In addition, restricted land resources is a crucial factor when considering food security (Sood et al., 2017). In this case potato proves to be the most promising productive crop for its considerable yield in terms of calories per acres compared to other major cereal crops. Not only in calories, but potato produces more energy and food value per unit of land relative to

any other significant crops. Hence, when dealing with future food production pressure, potato is the ideal crop.

Although there is an increase in potato production especially in Asia the growth rate have not been continuous, with slow growth with a few intermittent increases from time to time, and hence it is necessary to analyze the long term strategies to increase potato production (Scott and Suarez 2012). The strategies could range from research on breeding programs to consumption practices.

On top of aiding the food and nutrition security to the country, it is suggested that potato can be an attractive export earner to augment the Bangladesh's low income situation (Roy et al., 2017). In fact, the export of potato has been increasing recently and is all the more reason to invest time and resources to improve the country's potato production.

With numerous benefits including its nutritional content and the relative ease of cultivation, potato have been highly recommended by Food and Agricultural Organization (FAO) (FAO, 2009). In fact, the year 2008 was declared as the "Year of the potato" by the United Nations General Assembly, highlighting the importance of the food crop in the world. Notably, it has been quoted by the Director of the FAO, that "The potato is on the frontline in the fight against world hunger and poverty".

Since potatoes serve as a reliable food and nutrition security crop which can eventually contribute to the sustainable agri-food system (Devaux et al 2021), potato breeding research is a key interest in horticulture science.

In Bangladesh

Potato production have been increasing in developing countries since it is widely adaptable, relatively easy to cultivate (Devaux et al., 2014) and can be used as cash crops among farmers (Uddin et al., 2015). In Bangladesh, the production of potato is on average 11 Mt

making it the third-most potato producing country in Asia and 7th in the world ((Jannat, Ishikawa-Ishiwata and Furuya, 2021). In Bangladesh, it is the third largest food crop, after rice and wheat and it is cultivated in all its divisions (Roy et al., 2017). Potato is also the principal vegetable in Bangladesh (Devaux et al., 2020, pp.11). In fact, since the last six decades, there is an increase in the total production of potatoes together with the rapid rise in consumption in the country (Roy et al 2017). Similar to many other developing countries in Asia, potato consumption per capita in Bangladesh has increased; most likely due to the increment of incomes (Scott and Suarez 2012). The temperate climate of this country makes it favorable for cultivating potatoes (Jannat, Ishikawa-Ishiwata and Furuya, 2021; Uddin et al., 2015), and hence has immense potential in growing and sustaining its potato producing industry. Being an economically developing country with a growing population in addition to having a well-suited environment for growing potatoes, Bangladesh saw a significant expansion in potato production in the recent decades (Devaux et al 2021; Jannat, Ishikawa-Ishiwata and Furuya, 2021; Uddin et al 2015). On top of aiding the food and nutrition security to the country, it is suggested that potato can be an attractive export earner to augment the Bangladesh's low income situation (Roy et al., 2017). Moreover, potato production requires labor intensive approaches and hence Bangladesh, which has rising man to land ratio, is ideal in terms of its human resource requirements (Scott and Suarez 2012). In fact, the export of potato has been increasing recently and is all the more reason to invest time and resources to improve the country's potato production. Potato is an ideal crop to harvest as it is flexible in terms of adjusting to different crop rotation systems (Sood et al 2017).

Due to globalization, there has been a "nutrition transition" which probably have led to a shift towards a western diet in many countries (Devaux et al., 2021). This has increased the development of fast-food outlets, and supermarkets, hence there is an increase in demand for processed as well as prepared potatoes (Devaux et al., 2021). There has also been a rise in

income levels and urbanization which reflects in the increased affordability and acceptability of different type of potato-related western food compared to traditional food.

Considering both the population expansion and the increased popularity of potato-based food products, it can be predicted that potato production will be a key of interest in Bangladesh's future.

History of Potato

Potato cultivation has a rich history; from its Andean origin in South America, 8000 years ago, to the Europeans lands. And eventually by the 17th century, to the Asian continent (FAO, 2009). It is said that potatoes were brought over to India by the British missionaries in the late seventeenth century (Hawkes, 1992, pp.1-12). From being a crop gown only in South America, over the course of four centuries, it has now become one of the top producing crop worldwide (Hawkes, 1992, pp.1-12).

Potato played a crucial role in significant world events though the last four centuries. For instance, the food crop served as the primary food source when building the Inca Empire in the 13th century and by the 19th century, potato helped fuel the Industrial Revolution in Europe (Jansky, Navarre and Bamberg, 2019). The success in potato production is likely due to its efficiency. This crop could yield twice or more calories per acre than other grain crops (Connell, 1951; Jansky, Navarre and Bamberg, 2019). In addition, the labor required for the cultivation, including small farmers, could be easily sustained (Jansky, Navarre and Bamberg, 2019). In fact, with only a single acre of land for cultivation, together with a milk cow, could provide all the essential nutrients for a family of eight.

Importance of Research on potato breeding

With the rate at which the world population is growing, 70% more food will be required for consumption than is today (Devaux et al., 2021). Even the current food systems are not able

to supply adequate food that is nutritious in an environmentally sustainable approach (Wu et al., 2018). Although, it seems that worldwide there has been a progress in global hunger reduction, however, there are significant disparities at the regional, national and subnational levels (Devaux et al., 2020, pp.4). Moreover, the increasing urbanization of cultivable land pose a major threat to agriculturally important food crops (Govindaraj, Vetriventhan and Srinivasan, 2015).

Climate change is another crucial factor to take into account in terms of food security. It is expected that climate change may result in a significant decrease in the major cop production; such as rice production may decrease by 20-30% by 2100 (Jannat, Ishikawa-Ishiwata and Furuya, 2021). And hence, it is suggested by FAO that countries transition to more sustainable tuber crops particularly potatoes, due to its food and nutritional security).

Due to the uncertainties of climate change, there should be an emphasis on agriculture to initiate more productive, resilient and sustainable approaches for food crop production (Jannat, Ishikawa-Ishiwata and Furuya, 2021). Moreover, the food systems will need to i) generate adequate supply of nutritious food ii) maintain livelihood for farmers including small-scale farmers iii) maintain the environmental footprint at a minimum (Devaux et al., 2021). The aforementioned points are a challenge when approached simultaneously, since producing more food with the same or less resources may cause increased inequality or adverse effects on the environment. It should be noted that agriculture and food production should maintain sustainable usage of natural resources including soil and water.

Potatoes are usually grown locally, not like other major crops such as rice which are global commodities. Hence, it in relative terms, at global scale, potatoes are more resilient to price volatility as they are unlikely to be affected by political, financial or commercial factors (Campos and Ortiz, 2020). Potato is both essential for the fresh market and as the raw

material for French-fry/chip processing industry (Sood et al., 2017). The crop is used as both staple food and vegetables (Gebhardt 2013). It is also used for production of starch and alcohol. In Bangladesh, potato is the principal vegetable and is the third most important food crop (Devaux et al., 2020, pp 11).

To summarize, the reasons to work on potato research and innovation are (Campos and Ortiz, 2020); (i) there is an increase in demand for food due to the growing population in not only in Bangladesh but the world (Uddin et al 2015) (ii) provide food security and (iii) provide nutritional security (iv) aid in improving the economy (v) is a source of cash income (which is an essential requisite for food security) (vi) reduce post-harvest losses (vii) sustainable food choice in the face of climate change ramifications (Jannat, Ishikawa-Ishiwata and Furuya, 2021) . The increase in food demand will also need to combat with challenges that arise with a growing population such as (viii) decrease in and degradation of land and (ix) cultivating with less water resources (Uddin et al 2010). Moreover, (x) a lack of quality seeds was deemed one of the major problems potato growers faced during a 2000-2001 study (Uddin et al., 2010).

Nutritional Benefit

The nutritional benefits of potatoes are numerous and is, unfortunately, not widely known.

At least 12 vitamins can be obtained from potatoes (Carputo, Aversano and Frusciante 2004). The vitamin content of 100 grams of fresh tubers have 20 mg of Vitamin C which is half the intake amount required for us daily. In contrast, rice and wheat have no vitamin C. In fact, two fresh potatoes could provide the same amount of vitamin C as an orange, grapefruit or three apples (Lang 2001). Other vitamins potatoes are rich in are the B complex vitamins (e.g. thiamine and riboflavin). In fact, our body can efficiently utilize the thiamine from potatoes more than brown rice.

Potatoes also contain significant amount of a range of minerals especially including calcium, iron and potassium. In addition, it's low content of sodium, makes potatoes suitable for salt-free diets and aid with acidity issues.

Moreover, potatoes are low in calories, low in cholesterol and almost fat free (Lang 2001). In comparison, carrot have more fat than a potato.

The high content of starch in potato (Lang, 2001), makes potato a healthy source for energy. Once cooked, the starch is easily digested, and so it can account for a higher glycemic index (Jansky, Navare & Bamberg, 2019). Moreover, 95% of the carbohydrate in potatoes is digested by our body slowly, hence able to give us a steady supply of energy. The starch in potatoes, in addition to the fiber and anthocyanins are anti-inflammatory products, hence are responsible for enhancing gut health and decreasing chronic diseases.

Nutritionists have an appreciation for the crop due to its contribution to a balanced diet (Devaux et a., 2021). Indeed, potatoes is now considered as a functional food for athletes, as it fulfills their nutrient-dense and high quality carbohydrate requirements (Jansky, Navare & Bamberg, 2019).

Limitations in Potato Production

Despite the benefits as a food crop, limitations arise in potato harvesting process and hence the overall potato production (Maldonado, Wright and Scott, 1998). The challenges may arise in the form of insect pest or disease infestations (FAO, 2009b; Govindaraj, Vetriventhan and Srinivasan, 2015), expensive fungicides/pesticides, expensive fertilizer, limited storage facilities and limited supply of quality seeds (Uddin et al., 2010) which in turn will affect the yield of the crops

i) Quality of seeds is crucial in determining the yield since they are the main source for carrying diseases (Devaux et al., 2020, pp 17). Especially and most commonly,

viruses are the main culprit that cause seed degeneration which in turn leads to decrease in potato productivity. This is exacerbated with, the lack of certified seed been made available to farmers especially since farmers need to renew cultivating varieties according to the season and disease infestation (Muthoni and Nyamongo, 2009),

ii) Soil quality and environmental constraints can affect the yield in various ways (Manrique 1993). For instance, with high rainfall and thus water logging, there would be increased chances of disease infestation. Depending on the available nutrient and water, the plant growth can be affected (Manrique 1993) and thus an impact on the overall yield (Muthoni and Nyamongo, 2009). Moreover, due to heavy rainfall desiccating wind and other factor, quality of tuber may be affected (Kolech et al., 2015).

iii) Climate change is considered to exacerbate the challenges already present in potato production (Ellis et al., 2020, pp133). For instance, the duration for late blight infection will increase for each degree of warming which will in turn require longer need of fungicide application. Moreover, higher temperature may cause resistance genes to be ineffective. In addition, many pests (including nematodes, aphids and weevils) is influenced by a warmer climate. Besides the abiotic challenges, evidently the biotic stresses will increase, such as drought, heat and unseasonable weather (Ellis et al., 2020, pp 131)

iv) Potato is notably susceptible to various biotic stresses due to the fact that they are asexually propagated in their tuber form (Bhardwaj et al., 2019). Pests and diseases that results in potato production loss include:

a) Bacterial wilt, caused by *Ralstonia solanacearum*, is a major disease affecting potato production. At altitudes between 1800 and 2800m, this disease has reduced the yield ranging from 30% to 70% (Muthoni and Nyamongo, 2009).

b) Late blight is one of the most important disease affecting potato production. The oomecyte, *Phytophthora infestans*, causing the diseases has resulted in 10% to total crop failures (Kolech et al., 2015; Taylor and Dawson, 2021).

c) There are about five viruses that are notorious for significantly impacting potato production mainly by seed degeneration; Potato leaf roll virus V (PVV), Potato virus S (PVS), Potato virus X (PVX), Potato virus M (PVM), and Potato virus Y (PVY) (Taylor and Dawson, 2021). These virus infections

may affect the potato plant in several ways for instance, dwarfing of the plants and significant amount of yield losses.

d) Potato cyst nematode (PCN) (*Globodera* spp) are parasites which, even with a small infection, can cause severe and costly damages (Gartner et al., 2021). *G.rostochiensis* and *G.pallida* are accountable for the loss of 9% yield loss globally.

v) Costly investments for farmers while combatting the many of challenges related to potato production. This cause a minimal usage of expensive fungicides, fertilizers and low quality seeds (lack of beneficial or resistant traits), leading to low yield (Muthoni and Nyamongo, 2009). On the other hand, it was reported that when fungicides were applied to overcome late blight, around 13-14% of the total production cost was required.

These constraints regarding agronomic challenges, in the form of pests and diseases, establishes the need to develop potato varieties with quality traits which are disease/pest resistant, durable in the face of harsh climate and condition and overall high-yielding; in order to be profitable for breeders and farmers.

And hence the need for research is suggested in areas including: developing cultivar (genotypes) with abiotic and biotic disease resistance traits, high yield traits, earliness traits etc and other areas such as bio fortification of cultivars and high quality seed production (Devaux et al, 2020). Although biocontrol is still not widely successful, the research is nevertheless active and is expected to increase in the following decade.

It is also important to note that different varieties will grow differently depending on the area or region (Uddin et al., 2010) and this may be because a particular environmental condition may suit one variety compared to another. Hence, it is wise to keep a bank with wild types, modern cultivars and cultivars with various agronomic traits in every locality for breeders to have easy access.

To respond to the aforementioned challenges, the concept suggested by Haverkort can be applied which is the formula to determine the prospects of food security challenges: P= G × E× M× S (Devaux et al., 2020 pp. 20).

Here, P is Performance, and it relies on the Genotype or varieties (G); E is for the Environment where the crop is grown; Management or adaptation to the local socioeconomic conditions (M); Societal requirements that is the society's demand and environmental consumer friendly agriculture. Hence, we can see that there are many factors that will determine the performance of potato production. Genotypes of potato varieties is evidently a crucial factor. Environment E is a major issue since, as discussed before, the future of climate change is only excpected to exacerbate with unpredictable patterns. Meanwhile, the consumer requirement for potatoes for different purposes will factor in its performance as well. Finally, the food availability in terms of both quality and quantity for consumers in developing world is also of high importance. Eventually, to keep all the above factors in mind it is apparent for the need for research and innovation with the main goal to increase potato production with sustainable measures for farmers, breeder as well as consumers.

Traits

The aforementioned constraints hint to the urgency to develop potato varieties with quality traits such as high-yielding, abiotic and biotic stress tolerant traits (Slater et al 2014) in order to increase productivity for farmers. In fact, the most important objective for breeding programs, whether public or private, is the selection and distribution of cultivars with desired traits (Gebhardt 2013). As such, traits that will benefit farmers as well as industries and consumers need to be considered.

Agronomic traits that are key to cultivation are: high-yield, thick stem, early maturity, tuber size and number, long shelf-life, large plant height, number of leaves and sprouts etc.

especially for farmers (Govindaraj, Vetriventhan and Srinivasan, 2015; Kolech et al., 2015). Moreover, tuber yield and plant maturity (earliness) are crucial since they have shown to reflect profitability (Douches 1996).

Cultivars are developed based on their innate resistance to pests and diseases which would mean a reduced requirement of expensive pesticides and fungicides and hence deem economically beneficial (Bradshaw 2007, pp157-177). With severe epidemics caused by late blight which resulted in huge losses, in Europe and North America and the infamous Irish potato famine during the time of 1840s, cultivars with resistances to prevalent pest have been prevalent for a very long time (Bradshaw and Ramsay, 2005). Resistance traits include resistance against late blight (*Phytophthora infestans*), cyst nematodes (*Globodera rostochiensi* and *G. pallida*), *Verticillium* wilt (Bradshaw and Ramsay, 2005; Gebhardt, 2013). Resistance to biotic as well as abiotic stress, such as tolerance to drought, are highly prioritized for farmers.

Moreover, depending on the requirement of the market demand and the end uses, corresponding traits are selected for commercial viability (Douches et al 1996; Gebhardt 2013). For instance, varieties for culinary, chip-processing and tablestock potato purposes are selected for traits such as flesh/skin color and eye depth (Douches 1996). Dry matter of potatoes is a characteristic which is suitable for fresh-market consumption whereas low-reducing sugars and high specific gravity is suitable for chip-processing potato products. Potato tuber shapes is also a factor when determine utilization, for example, long-white or long-russet types are appropriate for frozen-processing and tablestock purposes. Evidently, the attractiveness in tuber appearance is also considered for marketability. With the growing potato consumption in the world, potatoes need to meet varying consumer demands (Sood et al., 2017). Hence, breeding potatoes need to consider selecting cultivars with quality traits depending on the specific market utilization i.e. the demands of the grower, processor and

surely the consumer. Based on the type of market need, the quality traits may be categorized as i) external quality: including skin colour, tuber size, shape and eye depth and ii) internal quality: including culinary value, properties retained after cooking and processing quality (Carputo, Aversano and Frusciante 2004). The latter quality is often a result from traits such as dry matter content, sugar and protein content and starch quality.

The overall tuber quality of potatoes can be influenced by various factors including agronomic practices, environmental conditions (e.g. temperature and presence of pests), and the genotype of the variety.

In fact, the most important factor that have influence on the quality traits would be the genetic make-up of the variety (Carputo, Aversano and Frusciante 2004). There are three categories that the traits controlled by the genetic make-up can be categorized in: i) Biological Traits, ii) Sensorial traits and iii) Industrial traits.

i) Biological traits include the proteins, carbohydrates, vitamin, minerals etc. composition of the tuber
ii) Sensoral traits include flavor, texture and colour of the tuber skin or flesh
iii) Industrial traits include size and shape of tuber, dry matter content, starch quality etc.

Generally important traits mainly related to yield, quality of tuber and stress tolerance (e.g. *Potato Virus X*, late blight, drought and salinity) are considered (Gebhardt, 2013) for cultivar development in breeding programs (Sood et al., 2017). In addition, specific traits related to particular production areas are also considered which could include: tuber shape, color of skin color of flesh, texture, eye depth, and possibility of tissue discoloration after cooking and likelihood of bruising (Gebhardt, 2013).

Importance of Genetic Diversity

The genetic variability that exists among individuals within a species is its genetic diversity.

Potatoes diversity is one of the factors contributing to the success of potato production (Arcimboldo, 2008). To obtain high-yield and desirable traits, it is a necessity for crossing of potato cultivars with a broader genetic base so as to increase the heterosis event (Mendoza and Haynes, 1974). Heterosis, mainly a consequence of genetically distant parents, give rise to increased beneficial traits such as growth rate, size, tolerance to various stress and overall productivity (Acquaah, 2020). Hence, breeders need to avoid selecting closely related parental cultivars in order to avoid inbreeding and maintain potatoes with high heterozygosity (Demeke et al., 1996). This is required for cultivar development for both fresh market use and French fries (Slater et al., 2014). A broader genetic base will thus ensure crossing of distinct potato cultivars during parental selection (Reddy et al 2018; Mendoza and Haynes, 1974; Wang et al., 2019).

Crops have notoriously, been the subject of severe pest attacks due to a narrow genetic base (Rahman et al., 2022), since a narrow base would mean a lack of useful genes for traits such as resistance against pathogens and diseases, overall hampering the breeding progress (Bradshaw and Ramsay, 2005). And so a diverse gene pool is crucial to solving issues related to abiotic and biotic stress during cultivation (Slater et al, 2014). The continuous changing conditions in the world and the unpredictable constraints that arise due to pests, diseases and various abiotic stresses in crops, are overcome by the genetic diversity found in the available plant genetic resources.

However, traditionally, breeders' cross varieties not based on their genetic makeup but though phenotype selection which may potentially cause repetitive cycles of inbreeding (Rahman et al., 2022). This practice if done continuously would significantly narrow the gene pool available. When using cultivars with a high degree of relatedness during breeding, yield would likely decrease (Mendoza and Haynes, 1974), susceptibility to pest attack would

incline together with other deleterious effects (Govindaraj, Vetriventhan and Srinivasan, 2015).

The infamous, Irish potato famine was caused by the over cultivation of genetically homogenous varieties (Govindaraj, Vetriventhan and Srinivasan, 2015). *Phytophtora infestans*, causing the late blight disease, was the main culprit of the famine that resulted in the death of one to two million people (Fowler and Mooney, 1990, pp.45). This epidemic resulted from the genetically limited potato cultivars that were being mainly gown in Europe. In fact, if it were not for the genetically distinct potatoes, found in Andes which had developed resistance to this disease, potato could not go on to become a major crop consumed today. Another similar famine, resulting from the same problem of lack of diverse potato cultivation, is the Southern corn leaf blight epidemic in the USA (Fowler and Mooney, 1990 pp.47). Many more epidemic of crop plants (including coffee, cotton and Indian rice crop) had occurred in the 18^{th} and 19^{th} century, and notably all these historical events were caused by genetic uniformity among the crops. And for every of these events, the rescue came from a genetically distinct variety that had eluded homogenization or from one of the wild variety. These systems driven famines and historically tragic events, raise the importance of plant genetic diversity (Govindaraj, Vetriventhan and Srinivasan, 2015).

Both food security and nutrition security (Devaux et al., 2014) is a major concern for the global food system due to the expanding population, urbanization and continuous decrease in cultivable land). Modernization of the world reflects deforestation, land degradation and increased environmental stress due to urbanization and climate change, which subsequently threatens the biodiversity of plants (Govindaraj, Vetriventhan and Srinivasan, 2015). As a result, agriculturally important plant species are being extinct at large-scale (Govindaraj, Vetriventhan and Srinivasan, 2015). Meanwhile, many potato farming communities have lost important cultivars (Acquaah, 2020, pp.127) since modern varieties may not be suitable for

many farmers with low income (Govindaraj, Vetriventhan and Srinivasan, 2015). This reflects to the threat to genetic diversity of potato varieties.

Moreover, from the aftermath of the green revolution that resulted in the replacement of indigenous crop plants (Brown 1983; Govindaraj, Vetriventhan and Srinivasan, 2015) there was a ramification of genetic erosion and even extinction of essential genes such as genes for primitive and adaptive traits Govindaraj, Vetriventhan and Srinivasan, 2015). Despite the rise of new improved cultivars, it is important to understand that over the past few decades many wild relatives have contributed to the adaptive traits of improved yield, quality and resistance (Ellis et al.,2020, pp114). Consequently, it is the responsibility of scientists to conserve the all the diverse potato germplasms for the future.

In short, there is long-time understanding that there is a need for genetic diversity in a crop in order to facilitate a sustainable crop production as well as conservation strategies (Govindaraj, Vetriventhan and Srinivasan, 2015; Kaila et al., 2011). Hence, there is a need for conservation of diverse varieties that include land races and traditional varieties, consisting adaptive and productive genes, for future agriculture to prosper (Govindaraj, Vetriventhan and Srinivasan, 2015).

Gene Bank

In order to maintain plant's genetic diversity, agricultural scientist have been conserving the genetic material resources in the form of Gene Bank (Govindaraj, Vetriventhan and Srinivasan, 2015). The continuous growing demand for meeting food supply hugely depends on the proper conservation and utilization of crop plants' diverse germplasm sources (Brown 1983). It is highly suggested that both public and private genetic resource organizations or plant breeders ought to protect germplasm sources in gene banks so as not to lose valuable varieties (Brown 1983).

By the late nineteenth century, potato breeding was being hindered by the narrow genic base. Therefore, gene banks were established with the objective of forming collections of potato varieties found locally and even worldwide. The main objective of the gene banks was to conserve the genetic materials and using it further to broaden the genetic base (Bradshaw and Ramsay, 2005). Majority of the gene banks preserve their material in the form of botanical seed forms (Ellies et al 2020, pp121) which include traditional varieties as well as modern improved ones. While there are in vitro collections found, there are only a few with the International Potato Centre being holding the most extensive collection.

Moreover, gene banks provide a reservoir for breeders to use immediately, however it also serves as a source for plant science research of cultivated and wild potatoes (Ellis e al 2020, pp 109).

Examples of renowned Gene banks: International Potato Centre (CIP) in Peru, Dutch-German Potato Collection (CGN) in the Netherlands, Potao collection of the Vavilov Institute (VIR) in Russia, US Potato Genebank (NRSP-6) in the USA and Commonwealth Potato Collection (CPC) in Scotland (Bradshaw and Ramsay, 2005). Many of the germplasms in these banks are collected from decades ago, for instance the VIR has varieties from 90 years ago, and hence without these collections they would be virtually lost today.

Gene banks have also allowed for the distribution of the necessary variety for breeders such as pathogen-free stock (Ellies et al 2020, pp127). Hence, they have various screening and treating methods to maintain the quality of the germplasm (Ellies et al 2020, pp127). Genebank of CPC for example, maintains germplasms in the form botanical seed which have been screened for the lack of diseases and other parameters that meet a certain standard for plant health (Bradshaw and Ramsay, 2005). Hence, the conserved genetic materials will enable breeder to capitalize on the available traits and alleles for increasing breeding

productivity. Since there is always a change in challenges that the breeders/farmers need to face, these gene banks serves as an asset for the future when new discovered and yet-undiscovered traits could be deployed (Ellis et al 2020, pp.121).

As a result, it is evident that there is global understanding of the importance of conserving the genetic diversity of potatoes. In fact, The International Treaty on Plant Genetic Resources for Food and Agriculture, was established with the objective to conserve, sustainably use and fair sharing of the benefits from the available genetic resources (FAO, 2012). In addition, The Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture, corresponding to the challenges identified by the Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture (a worldwide assessment under FAO), has prioritized the need for conservation and sustainable use of the global plant genetic diversity.

Importance of documentation of germplasm in gene bank

Although, there are more than 1750 gene banks worldwide, it is estimated that only about 25 to 35% of the accessions they hold collectively is genetically distinct (Ellis *et al.,* 2020, p 109). Meanwhile, accessions that are conserved ex situ have exceeded 7.4 million worldwide, and such increase has been large part accredited to exchange or unplanned duplication (FAO, 2010). And yet only less than 30% of the accessions are genetically different. Hence, the Commission on Genetic resources for Food and Agriculture (CGRFA) have ensured that FAO ought to periodically monitor the state of the global plant genetic resources for food and agriculture (PGRFA). One of the main objective targets prioritized by PGRFA is for the conservation of the plant genetic resources and its sustainable use (FAO, 2012). Even with the growing recognition and mainstreaming for the management and conservation of genetic diversity, more research and effort is required to meet both the demands for diversity in consumer's diet and also to combat the challenges for sustainable food production. Moreover,

with environmental challenges predicted to increase with climate change, a wider PGRFA will be required for farmers as well as breeders. Therefore, gene banks such as CIP not only maintains a collection of diverse cultivated potatoes but also information on their characteristics, morphology and genetic documentation (Bradshaw and Ramsay, 2005). Indeed, without proper data from the evaluation and assessment of the germplasm being made available, breeding programs will continue to only use a small percentage of the total germplasms of a gene bank (Brown 1983). To reduce prebreeding work for cultivar development and genetic improvement, breeders need comprehensive genetic information of germplasm and its diversity (Bered et al., 2005; Govindaraj, Vetriventhan and Srinivasan, 2015).

It should be noted that genetic diversity is crucial for determining the value of a germplasm (Sood et al., 2017). Hence, not only adequate documentation and characterization of all collections is a necessity but making it accessible is equally important (Ellis et al 2020, pp 126; FAO 2012). Proper documentation will reduce the chances of current accessions being exchanged within a collection which in turn causes the unwanted duplication (FAO 2012). In addition, with such genetic information, various taxonomic classification can be compared and verified using genomic differentiation (Govindaraj, Vetriventhan and Srinivasan, 2015).

Identification of cultivars

Accurate identification of potato varieties is of high interest as it enables proper assessment and as a result accurate information of the genetic diversity of a germplasm could be obtained. Since a huge number of accessions are being introduced continuously worldwide, making identification of each cultivar all the more essential to conserve cultivar integrity and proprietary rights (Kawchuck et al., 1996). As a result, accurate and reliable methods to

identify potatoes, with regards to their genotype and genetic distances relative to one another, is continuously sought after (Ghislain et al., 2004; Tillault and Yevtushenko, 2019).

Traditional methods of characterization of potatoes use morphological markers, that characterize based on phenotypic traits, which can be identified visually such as flower pigmentation and seed shape (Chimote et al., 2004; Govindaraj, Vetriventhan and Srinivasan, 2015). Relying on morphological data can be subjective, tedious and unreliable due to the ability for the traits to be influenced by environmental factors (Coombs, Frank and Douches, 2004; Tillault and Yevtushenko, 2019; Wang et al., 2019).

Although some traits might be easily detected by their phenotype, others are more complicated to be assed accurately (Gebhardt, 2013). Meanwhile, some traits may be destructive such as when checking for susceptibility to tissue discoloration caused by mechanical damage. Meanwhile, some traits are extremely tedious to screen for as they require large number production which would only be possible after years of production. And hence genotyping parent cultivars can augment the breeding process. As a result, the genetic information of germplasm in the gene bank is essential.

Another approach for identification is biochemical characterization, using isozymes to detect allelic variants of plant varieties though electrophoresis methods. Although, these markers also come with the disadvantages that there is only a limited number of available markers and that results may be affected by plant development (Govindaraj, Vetriventhan and Srinivasan, 2015).

In contrast to morphological markers, DNA markers are not dependent on environmental conditions and can be found in all plant tissues and are thus more stable and reliable in accurately identifying various potato lines (Govindaraj, Vetriventhan and Srinivasan, 2015; Rocha et al., 2010). In addition, they are not affected by pleiotropic or epistatic effects, hence

providing quality data (Govindaraj, Vetriventhan and Srinivasan, 2015). In fact, there have been a significant progress made in developing efficient DNA marker, which have resulted in the increase in our knowledge of the genetic sources (Kalia et al., 2011). DNA markers can be used for genotyping varieties as they can detect any alteration of DNA, caused by deletion, duplication, inversion or insertion (Govindaraj, Vetriventhan and Srinivasan, 2015). DNA markers have also been shown to be a cheaper and more reliable alternative to next-generation sequencing for analyzing genetic identity (Tiwari et al., 2013).

In short, accurate identification of potato varieties are important for (Tillault et al., 2019): (i) germplasm management, (ii) seed certification, (iii) new cultivar registration (iv) trademark purposes (v) property rights and (vi) aid parental selection in breeding programs.

There is a wide variety of DNA markers currently being used in breeding programs (Govindaraj, Vetriventhan and Srinivasan, 2015). These include markers like RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), amplified fragment length polymorphism (AFLP) and SSR (simple sequence repeat); all of which have shown abilities to detect and identify the genetic diversity of potatoes (Milbourne et al., 1997; Powell et al., 1996). RFLP is an example of hybridization marker whereas most of the rest (RAPD, AFLP, ISSR and SSRs) are PCR-based markers (Kaila et al., 2011).

There are many reports studying different molecular markers, their efficiency in determining polymorphism, the advantages and disadvantages, and other characteristics, in terms of analyzing and identifying the genetic relationships among potato cultivars (Govindaraj, Vetriventhan and Srinivasan, 2015; McGregor et al., 2000). However, most of these markers have shown to have limitations regarding the approaches and results of detecting the genetic diversity (Milbourne et al., 1998). For example, RFLP needs several days while also needing comparatively large amount of samples (Kawchuck et al., 1996). AFLP and RAPD markers

produce dominant markers. The lack of stringency is a major problem of RAPD method (Kawchuck et al., 1996) whereas AFLP required more labor and time (McGregor et al., 2000). Hence, DNA markers such as RFLP, RAPD and isozymes have been used for routine identification on a limited basis (Kawchuck et al., 1996).

SSR Markers

This paper will focus on SSR, which are microsatellites or STR (short tandem repeat) (Wang et al., 1994), that are short DNA (around 1-8bp) tandem repeat motifs found in plant as well as animal genomes (Govindaraj, Vetriventhan and Srinivasan, 2015; Milbourne et al., 1998; Powell, Machray and Provan, 1996). SSRs exhibit polymorphism among varieties based on the difference in number of repeat lengths which may arise due to DNA polymerase slippage during replication (Milbourne et al., 1997; Powell et al., 1996), from mutation (Govindaraj, Vetriventhan and Srinivasan, 2015), unequal crossing over, mismatch/double strand break repair or retroposition (Vieira et al., 2016). This difference in these repeat sequence give rise to allelic variation among crop species and thus is able to discriminate between genotypes (Ghislain et al., 2004). This allelic polymorphism at a particular locus can also contribute to linkage analysis and trait selection in breeding in addition to the identification of varieties (Milbourne et al., 1998).

SSRs, overall, are widely acceptable genetic markers consisting of many desirable characteristics. These attribute includes:

i) SSRs are highly abundant in plant genomes, and both tubers and leaves could be used for detection (Milbourne et al., 1998; Kawchuck et al., 1996).
ii) Only a small amount of DNA sample is required for starting the experiments (Powell, Machray and Provan 1996)

iii) They exhibit hypervariability (Kalia et al., 2011) and highly polymorphic (Milbourne et al., 1997). They were reported to have high allelic diversity per locus (Milbourne et al., 1997)

iv) These markers are co-dominant in nature (Milbourne et al., 1997).

v) These markers are multiallelic, hence they can exhibit multiple alleles per locus (Kalia et al., 2011).

vi) They are distributed throughout the genome, most of them being in the non-coding regions (Kawchuck et al., 1996)

vii) They have wide genome coverage (Favoretto et al., 2011) including genomes of organelles (Kalia et al., 2011)

viii) They have been shown to exhibit reproducible results in terms of generating polymorphic alleles using site-specific primers targeting the SSRs (Kawchuck et al., 1996; McGregor et al., 2000).

ix) Allelic profiles of each SSRs in vegetatively propagated crops remain identical in the long term and hence they demonstrate continued effectiveness over an extended period (Kawchuck et al., 1996).

x) As long as stringent conditions are maintained SSRs provide accurate results with only a small amount of sample (Kawchuck et al., 1996).

xi) Although it is initially costly to develop the markers, after the markers are available the process in inexpensive (McGregor et al., 2000).

xii) There are now alternative strategies to develop SSRs which is robust and cost effective. For instance, SSRs can be identified through already known RAPD amplicons (Kalia et al., 2011). Or the markers can also be transferred from related species since the sequences flanking the SSRs are usually conserved in closely related species (Provan, Powell and Waugh 1996; Tillault et al., 2019).

xiii) They have chromosome specific location (Kalia et al., 2011)

xiv) They are suitable for high-throughput genotyping (Parida et al., 2009)

In many cases SSR have been shown to be more preferable compared to other DNA markers (Favoretto, Veasey and Melo, 2011; Kawchuck et al., 1996; Milbourne et al., 1997, 1998). Hence, SSR markers are ideal for fast, reliable, accurate and provide a theoretically unlimited source of identification method (Kawchuck et al., 1996).

SSR have shown to be informative, in terms of studying the genetic diversity and for genotyping, in a wide range of plants such as tomatoes and soybeans as well as potatoes.

Table 1 | Previous genetic diversity studies done on a range of plant species, using SSRs.

Plant Species	References
Potatoes (*Solanum Tuberosum)*	Tillault et al 2019
Rapeseed *(Brassica napus ssp. Napus)*	Hasan et al., 2006
Rice *Oryza sativa* L.	Jasim Aljumaili et al., 2018 Singh et al., 2016
Peanut (*Arachis hypogaea L.)*	Tang et al., 2007
Soybean *(Glycine max (L.) Merr.)*	Wang et al., 2006
Maize	Legessee et al., 2007
Sweet corn *(Zea mays)*	Rupp et al., 2009
Tomato *(Solanum lycopersicum* L.)	Benor, S. 2008
Sugarcane *(Saccharum* complex)	Devarumath et al., 2012

To identify SSR markers, primers are designed that recognize the flanking regions of the SSRs, or sequences that contain short tandem repeats. By analyzing short repeat sequences from the potato sequence database, the flanking regions can be obtained and hence the primers can be designed accordingly (Provan, Powell and Waugh, 1996).

SSRs in potatoes

Initially, it was shown by Kawchuck et al., (1996), that SSR markers could successfully assess diversity in potato cultivars. Following this, numerous studies have used SSR primers/markers for genetic analysis in potatoes.

SSR technology were developed gradually (Ghislain et al 2004), with the first generation using specific repeat motifs in gene sequences for identification. One of the first studies using SSR on potatoes was conducted by Provan, Powell and Waugh (1996) where a single microsatellite was shown to distinguish 18 tetraploid potatoes. On the other hand, the second generation used enriched genomic library screening and database searches to characterize 112 SSR primer pairs, and incorporated them in potato linkage maps Milbourne et al (1998). Following this, Ghislain et al. (2004) identified and analyzed 156 SSR primer pairs in terms of their quality and polymorphism, including the sequences studied by Milbourne et al (1998). In the study of Ghislain et al., (2004), following extensive characterization, a user-friendly set of 18 SSRs were developed for informative genotyping in cultivated potatoes. This set of SSRs was named the PGI kit (potato genetic identification). These SSR were chosen based on their polymorphic information content (PIC) value, a wide genome coverage, a low copy number and an optimum amplification quality of products. This set was further extended and improved to 24 SSRs, in Ghislain et al., (2009), by fingerprinting 742 landraces with 51 SSRs. The PGI kit was made to serve a wide range of applications, such as, the genetic relationships among germplasm collections can be studied, genetic distances and gaps between varieties can be identified and eventually genetic mapping can be established. They can also help in building pedigree analysis and show SSR allelic diversity etc. (Ghislain et al., 2004). The kit also is suitable for using independently generated data while maintaining cumulative analysis (Ghislain et al., 2009). As a result, this set can serve as efficient markers for genetic diversity studies in potatoes.

SSR markers have been used in genetic diversity analysis in different potato cultivars from germplasms originating from around the world. For instance: Argentina (Ispizúa et al., 2007), Brazil (Rocha et al., 2010), Canada (Tillault et al., 2009), China (Liao and Guo 2014), India (Sharma and Nandineni, 2014) and USA (Bali et al., 2017).

Thesis objective

Since, genetic variability is crucial for plant breeding hence the information on germplasm diversity is not only relevant but a necessity for strategic planning in breeding programs (Bered et al., 2005; Reddy et al., 2018). Moreover, as mentioned before, conservation and documentation of plant genetic resources is a global concern, with FAO implementing PGRFA to ensure such documentation (FAO, 2010). A target for the FAO on PGRFA is for the sustainable use of the plant resources (FAO, 2012). And one of the approach for this sustainable use is by germplasm evaluation and molecular characterization, particularly "Expanding the characterization, evaluation and further development of specific collection subsets to facilitate use" (FAO, 2023). Meanwhile other approaches include crop diversification programs and developing strategies seed production and distribution. The documentation on the PGRFA is facilitated by the World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture (WIEWS) database, which has information on more than 5 million accessions provided from 115 countries, 17 regional centers and around 870 gene banks. Such database facilitates information exchange of the conserved germplasms. In Bangladesh, Advanced Seed Research and Biotech Centre (ASRBC) is one of the regional centers registered in the WIEWs database by the FAO. Hence, this study will evaluate the genetic diversity of potato accessions from the gene bank of ASRBC.

In this paper, we used 9 established primers, including some produced by the PGI kit, which have shown high levels of polymorphism and have been used for diversity analysis. These 9 SSR markers were used to analyze the genetic diversity among 62 potato varieties, with diverse agronomic and economically viable character traits, from ASRBC, ACI Limited, gene bank.

Chapter 2

[Material and Method]

2.1 Plant Materials

In this study, 62 potato accessions from ACI gene bank were assessed, including ACI developed materials and exotic varieties obtained from the Netherlands, UK, Thailand and Myanmar all of which were acclimatized in Bangladesh (Table 1). Accessions with various special traits that have agronomic and commercially viable benefits were obtained (Figure 1). The skin, flesh and color of tuber ranged red, yellow, white and various shades and combinations in between. Most of the tuber shapes were: oblong to long (30 accessions), oval and round (14 accessions). The plant heights of the potato plants ranged from 21 cm to 100 cm whereas the number of main stem/hill mostly ranged from 1 to 6 cm. The number of tuber/hill for most of the plant varieties were between 5 to 20, meanwhile their weight ranged from 200g to 600g. The yield of tuber of the plants were 26 to 45 Mt/ha. Thus, the accessions were diverse in terms of various beneficial traits that are phenotypic. Many of the plants taken in this study included high yielding characteristics, among which some were of indigenous and also modern potato variety. Some accessions were disease resistant such as late blight, PVY and PLRV varieties. Accessions that exhibit commercially viable traits were also used, such as varieties suitable for French fries and chips.

The leaf samples for these phenotypically diverse potato collection were obtained from plants grown in the ASRBC (Advanced Seed Research & Biotech Centre) greenhouse to conduct this study.

Figure 1│*Different variety of potato accessions used in the study.*

Table 2 │Phenotypic data of the 62 potato accessions used in this study

Acc. no.	PG	Origin	Plant Morphology		Tuber Morphology					Yield component			
			PH	NMS/H	SCT	FCT	ST	ECT	EDT	NT/H	WT/H	YT	Special Trait
1	ACI pakri 1	Bangladesh	70.6	5.2	Bright red	White	Oval flattened	Red	Deep	26.0	427.0	36.4	High Yielding IPV
2	ACI pakri 2	Bangladesh	47.0	3.6	Red with white	White	Round	White	Moderate deep	22.0	395.0	30.1	High Yielding IPV
3	ACI LBR Aster	Bangladesh	59.1	1.9	Red	White	Long	Red	Shallow	10.2	713.7	59.5	High Yielding LBR
4	ACI LBR Karez	Bangladesh	80.4	4.0	Light red	White	Round	Red	Deep	9.6	538.9	43.0	High Yielding LBR
5	ACI LBR Pakri 1	Bangladesh	90.0	6.3	Deep red	White	Oval flat	Red	Deep	17.0	565.0	34.1	High Yielding LBR IPV
6	ACI Fry Alu 1	Bangladesh	73.6	4.5	White	White	Long	White	Shallow	5.9	355.0	30.1	High Yielding Processing Type, Suitable for French
7	ACI Fry Alu 2	Bangladesh	95.0	4.2	White	White	Oblong to long	White	Shallow	10.5	505.0	38.4	High Yielding Processing Type, Suitable for French
8	ACI Fry Alu 3	Bangladesh	86.4	6.0	White	Yellow	Long	White	Shallow	6.0	420.0	35.0	High Yielding Processing Type, Suitable for French
9	Valencia	Netherlands	51.6	2.1	Yellow	Yellow	Oval to Long	Yellow	Shallow	7.3	660.0	42.9	Table and Industrial purpose
10	Cartagena	Netherlands	28.1	1.6	Red	Yellow	Oval to Long	Red	Shallow	10.8	488.4	40.7	Table and Industrial purpose
11	Alexia	Netherlands	58.5	1.7	Yellow	Light Yellow	Oval to Long	Yellow	Shallow	7.8	446.4	37.2	Table and Industrial purpose

Acc. no.	PG		Origin	Plant Morphology		Tuber Morphology					Yield component			
				PH	NMS/H	SCT	FCT	ST	ECT	EDT	NT/H	WT/H	YT	Special Trait
12	ACI Alu (Amora)	7	Netherlands	66.0	1.9	Yellow	Light Yellow	Oval	Yellow	Deep	7.7	624.0	37.7	Table and Industrial purpose
13	ACI Alu (Aromata)	8	Netherlands	32.9	3.2	Yellow	Yellow	Oval	Yellow	Shallow	8.3	572.0	31.6	Table and Industrial purpose
14	ACI Alu 14		Bangladesh	52.3	4.3	White	Yellow	Oblong	White	Shallow	13.8	633.0	42.2	Short Duration MPV
15	ACI Alu 15		Bangladesh	88.2	7.5	Red	Yellow	Round	Red	Moderate deep	22.1	467.0	31.1	Short Duration IPV
16	ACI Alu 16		Bangladesh	75.0	7.0	White	White	Round	Pink	Deep	32.8	540.8	42.9	Short Duration IPV, Long Shelf Life
17	ACI Alu 17		Bangladesh	72.1	8.2	Red with White	Yellow	Round	Red	Moderate deep	67.0	392.6	32.7	High Yielding IPV
18	ACI Alu 18		Bangladesh	65.0	3.0	White	Yellow	Round to Oval	White	Shallow	10.1	410.0	34.1	High Yielding MPV
19	ACI Alu 19		Bangladesh	86.9	4.4	Attractive red	Light Yellow	Long	Red	Shallow	19.6	382.0	32.0	Table Potato
20	ACI Alu 20		Bangladesh	54.6	2.3	Purple	Purple	Long	Black	Shallow	8.2	385.0	32.1	High Yielding Anti-oxidant rich
21	ACI Alu 21		Bangladesh	92.4	2.0	Purple	Yellow with marginal	Oblong	Purple	Shallow	6.3	383.4	31.9	High Yielding Anti-oxidant rich
22	ACI Alu 22		Bangladesh	64.0	2.3	White	White	Oblong	White	Shallow	6.8	492.0	41.0	High Yielding

Acc. no.	PG	Origin	Plant Morphology		Tuber Morphology					Yield component			
			PH	NMS/H	SCT	FCT	ST	ECT	EDT	NT/H	WT/H	YT	Special Trait
23	ACI Alu 23	Bangladesh	67.0	2.2	White	White	Round	White	Shallow	6.5	486.0	40.5	High Yielding
24	ACI Alu 24	Bangladesh	82.4	1.8	White with pink spot	Deep Yellow	Round	Red	Shallow	6.0	468.0	39.0	High Yielding Anti-oxidant rich
25	ACI Alu 25	Bangladesh	39.1	2.0	White	White	Long	White	Shallow	6.8	522.0	43.5	Short Duration MPV
26	ACI Alu 26	Bangladesh	70.9	2.1	White	White	Oblong	White	Shallow	4.8	506.0	40.4	Short Duration IPV
27	ACI Alu 27	Bangladesh	74.0	2.9	White	Light Yellow	Long	White	Shallow	4.8	519.2	41.0	Short Duration IPV
28	ACI Alu 28	Bangladesh	55.9	2.2	White	White	Round	White	Shallow	4.3	364.8	30.4	Short Duration IPV
29	PG 028	Bangladesh	55.9	2.2	White	White	Oblong	White	Shallow	4.1	378.0	31.5	Short Duration IPV
30	PG 011	Bangladesh	68.4	2.4	Reddish White	White	Long	Red	Shallow	3.0	370.0	30.8	Table Potato
31	PG 013	Bangladesh	66.8	1.7	Red	Yellow with marginal	Round	Red	Deep	5.6	422.2	35.2	High Yielding Anti-oxidant rich
32	PG 016	Bangladesh	55.9	1.2	White	White	Oblong	White	Shallow	3.1	234.0	31.5	Table Potato
33	PG 017	Bangladesh	54.6	2.3	White	Yellow	Oblong	White	Shallow	8.4	256.8	35.4	Table Potato

Acc. no.	PG	Origin	Plant Morphology		Tuber Morphology					Yield component			
			PH	NMS/H	SCT	FCT	ST	ECT	EDT	NT/H	WT/H	YT	Special Trait
34	PG 019	Bangladesh	66.2	6.6	White	Light Yellow	Oblong	White	Shallow	5.8	315.6	32.3	Table Potato
35	PG 020	Bangladesh	53.2	2.3	White	White	Long	White	Shallow	4.2	367.2	30.6	Table Potato
36	PG 026	Bangladesh	44.6	2.6	Red	White	Oblong	Red	Shallow	7.0	465.6	38.8	Table Potato
37	PG 029	Bangladesh	55.7	2.5	White	Yellow	Oval to long	White	Deep	4.7	370.8	30.9	Table Potato
38	PG 048	Bangladesh	59.1	3.2	White	Yellow	Round	Black	Shallow	5.6	397.2	33.1	Table Potato
39	PG 050	Bangladesh	57.3	2.9	White	White	Round	White	Shallow	4.9	376.8	31.4	Table Potato
40	PG 053	Bangladesh	49.6	1.7	White	White	Round	Black	Deep	4.2	360.0	30.0	Table Potato
41	PG 056	Bangladesh	42.7	3.1	Reddish White	White	Oblong	Red	Deep	5.9	428.4	35.7	Table Potato
42	PG 060	Bangladesh	82.4	1.8	Deep red	Pink	Oval to oblong	Red	Shallow	6.0	472.8	39.4	High Yielding Anti-oxidant rich
43	Sharposhona (PG 061)	UK	73.4	2.2	White	White	Oval	White	Deep	8.2	410.4	34.2	Cyst Nematode, PVY, PLRV Resistant
44	Axona (PG 062)	UK	64.7	2.9	Pink	White	Long	White	Shallow	6.9	423.6	35.3	Long Shelf Life

Acc. no.	PG	Origin	Plant Morphology		Tuber Morphology					Yield component			
			PH	NMS/H	SCT	FCT	ST	ECT	EDT	NT/H	WT/H	YT	Special Trait
45	Blue Danube (PG 063)	UK	47.8	1.8	Blue White	White	Long	White	Shallow	5.2	380.4	31.7	Cyst Nematode, PVY, PLRV Resistant
46	Lavender (PG 064)	Thailand	43.6	2.0	Yellowish White	White	Oval	Yellow	Shallow	4.7	364.8	30.4	Table Potato
47	R1 x D6-1	Bangladesh	50.1	3.2	Red	Yellow	Oblong to Long	Red	Shallow	6.8	373.2	31.1	Table Potato
48	R1 x D6- 3 (red)	Bangladesh	46.3	2.4	Red	White	Long	White	Deep	5.7	361.2	30.1	Table Potato
49	R1 x D6-3 (white)	Bangladesh	48.9	2.1	White	White	Long	White	Deep	5.1	387.6	32.3	Table Potato
50	R1 x D6-4	Bangladesh	42.6	2.3	White	White	Long	White	Shallow	5.3	372.0	31.0	Table Potato
51	R1 x D6-6	Bangladesh	44.3	1.9	White	White	Oval	White	Deep	4.8	360.0	30.0	Table Potato
52	R1 x D6-10	Bangladesh	41.5	2.8	Red	Yellow	Oval	Yellow	Shallow	6.3	376.8	31.4	Table Potato
53	C2-95	Bangladesh	63.8	1.9	Red	Yellow	Long	Red	Shallow	12.4	553.0	32.4	Table Potato
54	Bumrah	Myanmar	52.5	1.5	Pinkish white	White	Round	Pink	Deep	9.8	471.6	39.3	Table and Industrial purpose
55	BARI Alu-77(Sarpomira)	Denmark	41.5	4.1	Red	White	Long	Red	Shallow	8.8	646.0	35.0	Table and Industrial purpose

Acc. no.	PG	Origin	Plant Morphology		Tuber Morphology					Yield component			
			PH	NMS/H	SCT	FCT	ST	ECT	EDT	NT/H	WT/H	YT	Special Trait
56	BARI Alu-07 (Diamant)	Netherlands	58.4	5.0	White	White	Oblong	White	Shallow	10.9	633.0	37.8	Table and Industrial purpose
57	BARI Alu-08	Netherlands	53.3	4.5	Red	Yellow	Oval	Red	Shallow	9.7	405.0	35.0	Table and Industrial purpose
58	BARI Alu-25 (Asterix)	Netherlands	56.7	5.4	Red	Yellow	Long	Red	Shallow	10.3	686.0	39.8	Table and Industrial purpose
59	BARI Alu-54 (Musica)	Netherlands	53.3	4.5	Red	Yellow	Oval	Red	Shallow	9.7	405.0	35.0	Table and Industrial purpose
60	BARI Alu-29 (Courage)	Netherlands	47.6	3.2	Red	Yellow	Oval	Red	Deep	8.3	466.0	31.9	Table and Industrial purpose
61	BARI Alu-13 (Granola)	Netherlands	51.2	5.5	Yellow	Yellow	Round to Oval	White	Shallow	10.8	420.0	28.0	Table and Industrial purpose
62	BARI Alu-53	Netherlands	89.7	2.9	Red	Yellow	Round	Red	Deep	10.8	457.0	36.6	Table and Industrial purpose

Table 2 | Acc.no, Accession number; PG = Potato Germplasm; PH = Plant Height (cm); NMS/H= Number of Main Stem/ Hill; SCT = Skin Colour of Tuber; FCT = Flesh Colour of Tuber; ST = Shape of Tuber; ECT = Eye Colour of Tuber; EDT = Eye Depth of Tuber; NT/H= Number of Tuber Hill; WT/H = Weight of Tuber/ Hill (g); YT = Yield of Tuber (Mt/ha); ST = Special Trait; IPV = Indegenous Potato Variety; MPV = Modern Potato Variety; LBR = Late Blight Resistant

2.2 DNA Extraction

Young leaf samples for each variety were collected from a single healthy-looking 15 day-old plant, weighing around 100-150 mg.

The equipment and reagents used are:

Table 3 | Equipment and reagents used for DNA Extraction

Equipments	Reagent
• Mortar and pestle • Incubator (Water bath 65ºC) • Centrifuge machine • Balance • pH meter • Autoclave machine • Distilled water plant • Water de-ionizer • Refrigerator • Eppendorf tube • Eppendorf tube rack • Micropipettes • Pipette tips • Beaker • Measuring cylinder • Conical flask • Weight machine • Magnetic Stirrer • Vortex machine • Measuring cylinder • Laminar air flow • Gel Electrophoresis System • Gel Documentation System • UV Spectrophotometer • PCR tubes • PCR machine • Fume hood	• 1M Stock Solution of TrisHCl (pH8.0) • 0.5 M Stock Solution of EDTA (pH8.0) • 5 M Stock Solution of NaCl • 5% Stock Solution of SDS (Sodium Dodecyl Sulphate) • 10% Stock Solution of PVP (Polyvinylpyrrolidone) • 20% Stock Solution of CTAB (N,N,N Cetyltrimethyl Ammonium Bromide) • Stock Solution of TE buffer (pH 8.0) • Chloroform:Isoamyl Alcohol (24:1) Solution • Isopropanol • 70% Ethanol • Extraction buffer: TrisHCl, EDTA, NaCl, CTAB, Sodium Sulphate, ddsH2O • 10X TBE buffer (pH 8.3): Trizma base,

DNA extractions of the samples were carried out using a modified CTAB method by Raihan et al. (2016) which includes grinding the roots in a mortar pestle using extraction buffer and then adding 10% PVP, 20% CTAB, 5% SDS. Following mixing the solution by inversion, the mixture was incubated at 65℃ in a water bath, for 40 minutes. During this time the mixture solution was further mixed intermittently by inversion. The samples were then cooled to room temperature and an equal volume of Chloroform: IsoamylAlcohol (24:1) was added and mixed by inversion. The mixture was centrifuged for 20 min at 12000 rpm using a Hitachi centrifuge (Type: CT15E, Hitachi Koki Co., Ltd), and the supernatant was discarded and an equal volume of ice cold isopropanol was added again following another round of centrifugation with 12000rpm for 10 minutes. The supernatant was then transferred and mixed together with 150µl of NaCl and 500 µl of isopropanol. After mixing the solution by inversion, a cotton-like DNA was observed. For complete pellet formation the mixture was stored at -20℃ for 1 hour. The DNA was washed by centrifugation, after adding 70% ethanol, at 12000rpm for 10min and then discarding the supernatant. Final DNA samples were obtained by drying the pellet completely prior to adding 50µl DEPC Treated water, and storing it at -20℃.

The isolated DNA quality was checked though 1% Agarose gel electrophoresis.

Finally, crude DNA samples were quantified by measuring the absorbance at 260nm using the 4802 UV/VIS Double Beam Spectrophotometer (UNIQO) in order to make a final working solution with a 50ng/µl concentration which would later be used in the PCR process for SSR amplification. The concentration of each DNA samples were multiplied to a constant (Conversion factor). Measurement at 260nm was then divided by the absorbances at 280nm. This ratio indicates the purity of the samples since a ratio is ensured to be at 1.8-2mm. (A ratio higher than 1.8 generally indicates protein contamination).

Calculation of DNA sample concentration:

DNA concentration = Abs260nm ×Dilution factor ×Conversion factor

$$= Abs260 \times \frac{\text{Volume of Nuclease free water (µl)}}{\text{Amount of DNA}} \times \text{Conversion factor}$$

- (µg/ml)

- (ng/µl) [since 1µg = 10^3ng i.e.µg/ml = ng/µl]

Here,

Abs260 = Spectrophotometric Absorbance at 260 nm of the DNA sample.

Dilution factor = the Ratio of the volume of Nuclease free water (µl) to the amount of DNA sample (µl)

Conversion factor = 50µg/ml of DNA contained in a solution gives Spectrophotometric absorbance reading at 260nm equal to 1.

2.3 SSR primers

Nine specific SSR markers were selected (Table 2) based on their high PIC values and discriminatory power between potato cultivars and are established by previous studies (Ghislain et al., 2004; Ghislain et al., 2009; Kandemir et al., 2010; Tillault and Yevtushenko, 2019; Tiwari et al., 2013). Information on marker names, primer sequences, annealing temperatures, location on chromosome are summarized in Table 2.

Table 4 | Primer sequence information for the 9 SSR markers used for the genetic diversity analysis

SSR marker	Primer sequences (5' to 3')	Chromosome number	Motif	Reference
STM 0030	F: AGAGATCGATGTAAAACACGT R: GTGGCATTTTGATGGATT	XII	(GT/GC)(GT)8	Kandemir et al., 2010; Tillault Yevtushenko, 2010
STM 0031	F: CATACGCACGCACGTACAC R: TTCAACCTATCATTTTGTGAGTCG	VII	(AC)5…(AC)3 GCAC (AC)2 (GCAC)2	Kandemir et al., 2010; Tillault Yevtushenko, 2010
STM 0037	F: AATTTAACTTAGAAGATTAGTCTC R: ATTTGGTTGGGTATGATA	XII	(TC)5 (AC)6 AA (AC)7 (AT)4	Kandemir et al., 2010; Tillault Yevtushenko, 2010
STM 1016	F: TTCTGATTTCATGCATGTTTCC R: ATGCTTGCCATGTGATGTGT	VIII	(TCT)9	Tillault Yevtushenko, 2010
STM 1049	F: CTACCAGTTTGTTGATTGTGGTG R: AGGGACTTTAATTTGTTGGACG	I	-	Favoretto, Veasey and Melo, 2011
STM 1052	F: CAATTTCGTTTTTTCATGTGACAC R: ATGGCGTAATTTGATTTAATACGTAA	VII	(AT)14 GT (AT)4 (GT)6	Kandemir et al., 2010; Tillault Yevtushenko, 2010
STM 1106	F: TCCAGCTGATTGGTTAGGTTG R: ATGCGAATCTACTCGTCATGG	X	(ATT)13	Kandemir et al., 2010; Tillault Yevtushenko, 2010
STM 2013	F: TTCGGAATTACCCTCTGCC R: AAAAAAAGAACGCGCACG	VII	(TCTA)6	Kandemir et al., 2010; Tillault Yevtushenko, 2010
STM 3009	F: TCAGCTGAACGACCACTGTTC R: GTTTGATTTCACCAAGCATGGAAGTC	VII	(TC)13	Milbourne et al., 1998

2.4 Amplification of SSR

The DNA extracted from the 62 varieties were then amplified using PCR for each of the 9 markers with a total reaction volume of 10µl for the PCR mixture. The 10µl PCR cocktail was made up of 1µl DNA sample (for a 50ng/µl DNA template); 5µl of 2X pre-mix (EmeralAmp GT PCR Master mix), containing DNA polymerase, optimized reaction buffer, dNTPs, a density reagent, and green dye; 0.5µl of each primer (forward and reverse); and 3µl nuclease free H_2O.

For each primer set, 62 samples were then amplified on a thermal cycler (Veriti 96 well Thermal Cycler, appliedbiosystems by Thermo Fisher Scientific) following the program of: 95℃ for 5 min, 40 cycles of 95℃ for 20s, annealing temperature for the corresponding primer for 20s, 72℃ for 45s, and the final extension step was done at 72℃ for 10 min. The amplified products were stored at -4℃.

Precautions for PCR amplification:

1. PCR Eppendorf tubes and tips, used for PCR Reactions, were autoclaved
2. Mastermix was vortexed and centrifuged down before the PCR reaction.

2.5 Gel Electrophoresis

Equipments:

1. Gel Electrophoresis system:
    - Electrophoresis chamber
    - Gel casting Glass slides
    - Gel casting Glass slides holder
    - Comb
2. Gel Documentation

Reagents:

1. Agarose powder: To make agar gel
2. Tris-borate-EDTA(TBE) 10X Buffer
3. Ethidium Bromide: Dye, to stain the DNA, in order to observe bands
4. Bromophenol Blue (Loading Dye): Dye, to visualize the DNA traveling through the gel

Nucleic Acid and Oligonucleotides:

1. 100bp DNA Ladder
2. DNA samples (Obtained from PCR)

Stock solutions for Gel Electrophoresis:

Stock solutions required for Gel Electrophoresis is were prepared using the following processes:

10X TBE Buffer (pH 8.3):

The reagents required for making 10X TBE buffer are: Trizma base, EDTA, Boric acid, NaOH, sterile distilled water (sddH_2O). To obtain 10x TBE Buffer in 100 ml the following weight was taken for each:

Trizma base (MW 121.1) : 10.89 gm

sddH_2O : 75 ml

EDTA.2 H_2O ((MW=372.2) : 0.93 gm

Boric acid : 5.5 gm

Method:

First, 10.89gm of Trizma base was dissolved into 75ml of sddH_2O. Then, 0.93 g of EDTA.2 H_2O was added to the solution before adding 5.5gm of Boric acid. The pH of the solution was adjusted to 8.3 by adding concentrated NaOH (10 N). The final volume of the mixture was adjusted to 100 ml.

1.4% Agarose Gel Preparation

50 ml of 1.4% Agarose gel was casted for Electrophoresis (done in the later steps) following the steps: Firstly 1.4g of Agarose powder was weighed out and placed in a 250ml conical flask. Next, 20 ml of (1xTBE) buffer was added to the flask before wrapping the flask with aluminum foil. The following table shows the summary of the components used.

	Working concentration for 70ml Agarose	Amount
Agarose	2%	1.4g
10XTBE	1XTBE	70ml

The Flask with the mixture is then heated in an oven using a low to medium setting for 2 mins. After heating, the flask is gently stirred and swirled to dissolve the agarose that still seemed undissolved, and this was done until all the translucent agarose was dissolved. The flask was then set at room temperature until it was cool enough to hold with bare hands i.e. around 50^0C. From a 10 mg/ml concentration of ethidium bromide solution, 2 µl was added to the flask and gently swirled to mix well. EtBr is required to make DNA visible under UV light. And the final concentration of EtBr in the mixture solution will be around 0.5 - 1.0 µg/ml.

Comb Set-up

The gel casting glass slides were set up using the glass slide stand, which has clips to hold the slides in place. The molten agarose (around 50℃) was poured onto the top opening slot, created by the small gap in between the slides, by using a 5ml pipette. Immediately, the comb was set on the top slot of the glass slides, at the opening in between the slides. The gel was allowed to solidify for 40 minutes at room temperature.

Once solidified, the comb was gently removed from the glass slides.

Notes: Hot Agarose (60℃) was not poured immediately into the glass slides, as it might warp the plastic comb and hence decrease its lifetime. Warping might also result in uneven depth wells.

Any air bubbles should be moved aside, using a disposable tip, to avoid irregular wells.

Preparing DNA sample for Electrophoresis:

The DNA samples in the eppendorf tubes from the PCR reactions, were placed on ice. In each tube, 2µl of loading dye (Bromophenol Blue) was added and mixed with a pipette.

Electrophoresis

The gel casting slides with the solid gel were set in place vertically, at the center of the electrophoresis chamber. 1 X TBE Buffer was added to the center of the chamber until the gel was immersed sufficiently, however the volume should not be above the red line marked in the tank.

Loading DNA samples in wells

The total volume of the loading sample was set to be around 10µl, and this volume was decided depending on the size and depth of the wells. Each sample (mixed with the loading dye) was then loaded into the wells. In either sides of the samples, or in both ends, 2µl of a 100bp DNA ladder (molecular weight marker) was also added into the wells.

When pipetting during the loading process, the tip was placed in the center of the wells when pouring the DNA sample, and the dye was observed until it sets to the bottom of the well, before taking the pipette away.

Setting up Electrophoresis tank

The cover of the electrophoresis chamber was placed on the correct side, by checking the red and black marks on the cover and in the tank. Once in place, the electrophoresis machine was turned on and the Voltage was set to 100V for 50 minutes. The migration of the dye, which corresponds to the separation of the DNA, was monitored. The movement of dye and DNA should be downwards towards the anode.

When the dye reaches 3/4th of the length of the gel, i.e. towards the bottom end of the tank, the electrophoresis was stopped using the "stop" button on the machine.

Documentation of the DNA samples from gel electrophoresis

The casting glass slides were carefully taken out from the electrophoresis chamber, and placed on a tissue on the bench. With the help of a scalpel the glass slides were separated. The gel together with the glass slide it was on, was placed in a container of buffer solution mixed with EtBr. The container was covered and was allowed to rest for 15 minutes.

The gel was then separated from the glass carefully and placed on the Gel Documentation system, UVsolo TS Imaging System, by Biometra. The DNA bands were observed under UV light, by turning off the lights in the lab.

Bands on the gel were imaged by the gel documentation system, and their sizes and patterns were recorded.

Precautions:

EtBr is a carcinogen and a mutagen, hence this reagent was handled with extreme care when using it or any equipment that were exposed to it. Gloves were used when handling the EtBr reagent. Any gloves used and the gel that were needed to be disposed were disposed of in an identified bag which were in turn disposed of in an identified plastic bucket. These were identified so that all EtBr containing waste was discarded in this bag, which could then be sent to the incineration. Any remaining equipment, such as the glass slides and scalpel were washed thoroughly with tap water.

Since the gel documentation system produces an UV of 254 nm range, eye protector was worn to avoid eye damage, Lab coat and gloves were worn to protect the skin.

Troubleshooting Electrophoresis problems

The time and voltage that the electrophoresis is run with is important in terms of the band quality obtained. Hence they were decided upon carefully. With a low quality DNA band observation, the run time, voltage and even temperature could be adjusted slightly to optimize the band images.

2.6 Data analysis

Allele detection and size measurement was done using Image Lab Software (v.6.0) (Tillault and Yevtushenko, 2019). Allele counting was done in an approach using 1 as present and 0 as absent at the same mobility, and hence total allele number was determined for each SSR amplified product (Liao and Guo, 2014).

The information content regarding allelic diversity for each SSR loci was determined from the polymorphic information content. For each SSR the PIC was measured using the following formula:

$$\boldsymbol{PIC = 1 - \sum p_i^2}$$

where p_i^2 is the relative frequency of i^{th} allele of the SSR loci (Nei, 1973).

By using the Past software (version 4.03), the 0 - 1 allele scoring results were recorded, from all the SSR amplified products, and subsequently the Jaccard's similarity coefficient was calculated and recorded in a matrix. This matrix was then used to construct a dendrogram using the unweighted pair-group method with arithmetic average (UPGMA) cluster analysis. Clustering of accessions was recorded.

Using the dendrogram generated by each separate SSR marker, the rate of distinguishing cultivars by cluster (RDCC) was calculated by the following formula:

$$\boldsymbol{RDCC = \frac{N - N_i}{N}}$$

here, N is the total number of cultivars evaluated, and N_i is the number of cultivars that remained indistinguishable (Liao and Guo, 2014).

Chapter 3

[Results]

3.1 SSR marker detection

In this study, DNA fingerprinting was done on a total of 62 potato samples using 9 SSR markers. The efficacy of the separation of amplified DNA products by SSR markers was ensured by first using primer set STM0031 and resolved on a 6 % polyacrylamide gel (Figure 2).

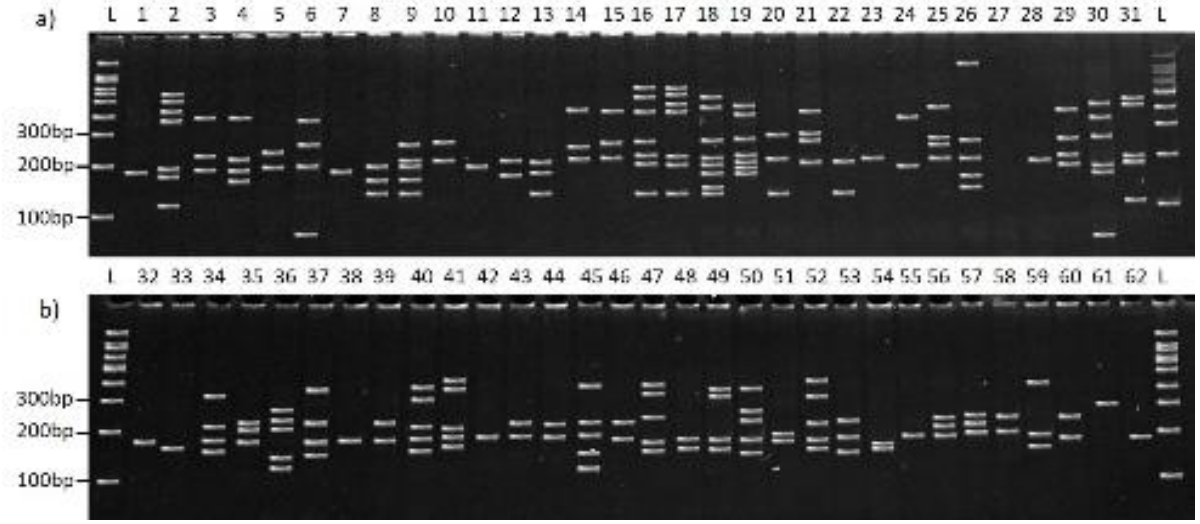

Figure 2| Polyacrylamide Gel Electrophoresis result of amplified products, using SSR marker STM0031, of the 62 potato accessions; (A) accessions 1-31 (B) accessions 32-62

3.2 Discriminatory ability of SSR markers

The size of the PCR amplified products, using the 9 SSR markers, ranged from 95 to 800 bp (Table 3). The number of alleles produced per marker differed from 3 to 11 (Table 4), while the average was 7.3. The fewest was produced by the primer pair STM1052 while the highest bands were detected by STM0031 primer pair. In total, 46 unique alleles were obtained from the PCR amplification using the 9 sets of SSR primers. Among the unique alleles, three were

monomorphic and the rest 43 alleles were polymorphic where the polymorphism percentage was 93.48%.

Potato Accesion Number	STM0030	STM0031	STM0037	STM1016	STM1049	STM1052	STM1106	STM2013	STM3009
1	210/152	390/205	380/180/98/95	770/680/550/490/430/270	440/370/200		165	800/350/300/190	420/400/150
2	250/210/152	550/440/390/205/120	180/98/95	770/270	440/370/200	220		300/190	150
3	280/210/185/152	205	98/95	770/270	440/370/270/200	220		190	420/400/150
4	420/360/280/250/210/185/152	205	540/380/180/98/95	680/550/270	500/440/370/270/200	250/220	165	800/190	420/400/150
5	250/210/152	205	380/180/98/95	680/550/490/430/410/270	500/440/370/270/200	250	165	800/190	420/400/150
6	152	550/520/440/390/350/280/205	380/180/155/98/95	770/680/550/490/410/270	500/440/370/270/200	460/250/220		800/350/300/190	420/400/150
7	360/280/250/210/185/152	240	98/95	680/550/490/270	270/200	220		190	420/400/150
8	360/280/250/210/185/152/130		540/480/380/180/98/95	490/270	500/270/200	460/250/220		800/420/300/190	400/380/175/150
9	280/250/152	550/440/350/280/205/175/140	500/98/95	680/550/490/370/270/180	440/370/200	220		300/190	380/150
10	250/152	240	540/480/380/180/155/98/95	800/680/550/490/430/410/370/270/180	500/440/370/270/200	220		800/420/300/190	420/400/290/150
11	250		98/95	800/680/550/490/430/410/370/270/180	440/370/200			300/190	400/380/290/150
12	420/280/250/210/185/130		380/180/98/95	410/270	500/440/370/270/200	460/250/220	800/165	800/420/300/190	400/380/150
13	152		380/180/155/98/95	680/550/490/430/270	500/440/270/200	460/220	800/230/165	800/350/300/190	150
14	360/210/185	440/205		680/550/490/270	200			300/190	420/400/380/175/150

15	360/280/250/210/185/152/130	440/280/240/205	380/180/155/98/95	680/550/490/430/270	500/440/370/270/200	460/250	800/700/340/290/230/165	800/420/300/190	420/400/380/150
16	250/210/152	550/520/440/390/350/240/140	380/180/155/98/95	680/550/490/430/270	440/370/200		165	800/300/190	290/150
17	250/210/152	550/520/440/390/350/140	155/98/95	680/550/490/410/370/270/180	440/370/200	220		300/190	290/150
18	152/130	440/280/205/175/140	380/180/155/98/95	430/270	440/370/200	460/220	230/165	300/190	400/380/290/175/150
19	250/185/152	520/440/390/350/280/240/205/175	380/155/98/95	800/770/680/550/490/410/370/270/180	200	460/220	165	300/190	420/400/380/290/150
20	250/152	390/280/240/140	380/155/98/95	800/490/410/370/270/180	270/200			300/190	420/400/380/150
21	420/250/210/185/152/130	550/520/440/390/350/280/205	380/180/155/98/95	550/490/430/270	500/440/270/200	460/220	800/700/290/230/165	800/300/190	400/380/150
22	250/210/185/152/130	240	380/180/155/98/95	800/680/550/490/410/370/270/180	500/270/200		800/700/490/290/230/165	420/300/190	400/380/150
23	152		380/180/95	770/680/550/490/430/270	500/270/200	220		420/190	400/150
24	360/280/250/152/130		95	410/370/270	440/370/270/200	220		800/190	190/150
25	360/210/185	440/350/280/205	180/155/98/95	680/550/490/430/270	200	220	165	350/190	420/400/290/175/150
26	250/152	440/240/175/140	98/95	800/680/550/490/410/370/270	440/370/270/200	460/220		350/300/190	175/150
27	280/250		98/95	680/550/490/430/410/370/270	270			350/300/190	400/175/150
28	250		98/95	770/680/550/490/430/410/370/270/180				190	400/175/150
29		440/280/205	98	770/680/550/490/430/270				300/190	400/175/150

30	420/360/280/250/210	440/350/280/240/205/175	180/98/95	770/680/550/430/270	440/370/270/200/	220	165	800/350/300/190	400/380/175/150
31	210/185/152/130	440/390/350/240	98/95	770/680/550/430/270	440/370/200	250		800/420/300/190	380/290/175/150
32	210/152		380/180/95	770/550/490/430/270	270/200			350/300/190	150
33	280/152	175	380/180/98/95	680/550/270	200		165	700/190	150
34	250/210/152/130	175	98/95	680/550/270	270/200	220	165	700/350/300/190	400/380/150
35	250/152	205/175	95	680/550/270		460/250/220			400/380/150
36	420/280/250/210/152/130	280/205/175/140	380/180/98	800/680/550/270	440/370/200	250	230	300/190	400/380/150
37	420/250/210	280/240/205/175/140	98/95	770/680/550/270	270/200	460/250/220		190	400/150
38	420/152	240	380/180/95	680/550/270	440/370	460/220	165	190	400/150
39	420/250/210/185/152/130		180/95/	680/550/430/410/270/180	270/200	460/250/220	165	700/350/300/190	400/380/150
40	420/280/250/210/185/152/130	390/350/280/205/175/140	180/95	800/680/550/430/410/270/180	270/200	220	800/700/340/230/165	700/190	400/380/150
41	210/152	390/350/240/175	180/95	680/550/270	270/200	220	165	350/300/190	400/380/150
42	420/280/250/210/185/152/130		95	430/270	270/200	250	290/230/165	190	400/150
43	280/250/210/185/152/130			680/550/430/410/370/270	270/200	460/220	165	190	290/150
44	420/280/250/152		180/95	680/550/490/430/410/270/180	500/270/200	460/220	800/700/340/165	700/420/190	290/150

45	280/250/210/152	175/140/120	180/95	680/550/490/430/270	270/200	250		350/300/190	400/380/150
46			540/500/480/380/180/155/95	680/550/410/270/180	500/440/270/200	460		700/420/350/190	400/150
47	152	350/175	95	680/550/430/270	200		230	300/190	150
48		175		680/550/430/270	440/370/200	250/220		300/190	400/150
49	280/250/152	440/390/350/280/240/205/175/140	98/95	490/430/270	440/270/200	460/220	165	350/300/190	400/380/290/190/175/150
50	152	280/240/205/175/140	180/98/95	680/550/270	200	460/220	800/700	350/300/200	400/380/190/175/150
51	280/250/152	440/175	380/180/98/95	770/680/550/430/410/270/180	500/440/370/270/200		800/700/340/230/165	800/420/350/300/200	400/380/150
52	280/250/210/152	175	98/95	680/550/430/270	200	460/220	800/700	300/190	400/150
53	280/250/210/185/152	240/205/175	380/180/98/95	770/680/550/490/430/270	440/370/270/200		490/340/165	300/190	380/290/175/150
54	420/360/280/250/210/185/152	175/140	380/180/98/95	800/770/680/550/490/410/270	500/270/200	220	490/340/290/165	700/350/300/190	400/380/175/150
55	152		380/180/98/95	770/680/550/270	500/270/200	460/220	165	700/350/300/190	380/150
56	250/210/152	240/205		680/550/270	270/200	220		350/300/190	175/150
57	152	240/205	180	270	200	460/220	230		150
58	152	240/205	380/180/98/95	800/680/550/490/430/270	270/200		165	700/350/300/190	175/150
59	152	175/140	98/95	800/680/550/270	270/200		165	350/300/190	400/380/175/150

60	152		98/95	270	270/200			350/300/190	400/380/175/150
61	420/360/280/152		380/180/98/95	680/270	440/370/270/200	220		190	150
62	152		180/98	270	440/370/200	460/250			150

Table 5 | Observed band sizes (in bp) of the PCR products amplified by each of the 9 SSR primers, for the 62 Potato accessions

Table 6 | Allelic information and PIC values obtained from the DNA samples amplified by the 9 SSR markers.

SSR marker	Expected Size (bp)	Observed band size (bp)	An. Tm	No. of Alleles	PIC
STM 0030	147	134-412	45.85	8	0.839
STM 0031	172	146-551	54.28	11	0.888
STM 0037	90	95-535	41.6	8	0.789
STM 1016	247	180-809	51.12	10	0.867
STM 1049	195	203-507	53.37	5	0.770
STM 1052	248	228-460	47.75	3	0.621
STM 1106	156	165-800	50.75	7	0.769
STM 2013	160	193-800	49.5	7	0.760
STM 3009	110	150-412	58.9	7	0.781

PIC, polymorphic information content; An. Tm, Annealing Temperature for PCR reaction

The polymorphism information content (PIC) by the 9 SSR markers, differed from 0.620 to 0.888 by STM1052 and STM0031 respectively. The mean PIC value obtained is 0.787 per SSR.

For each SSR marker, a dendrogram was produced of the 62 accessions (Figure 3-11). None of the markers, individually, were able to distinguish all the 62 accessions.

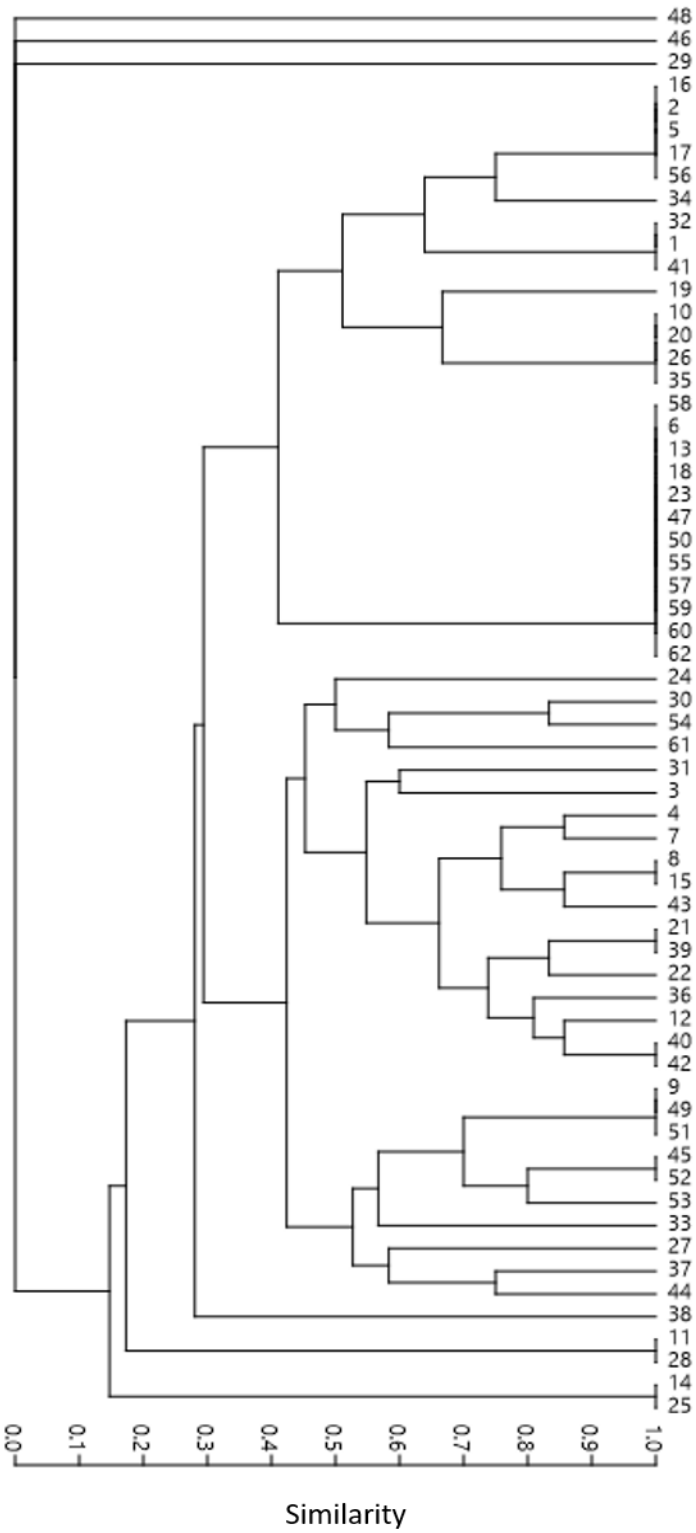

Figure 3 | Dendrogram of the 62 potato accessions based on their Jaccard's similarity coefficients and UPGMA, using STM0030

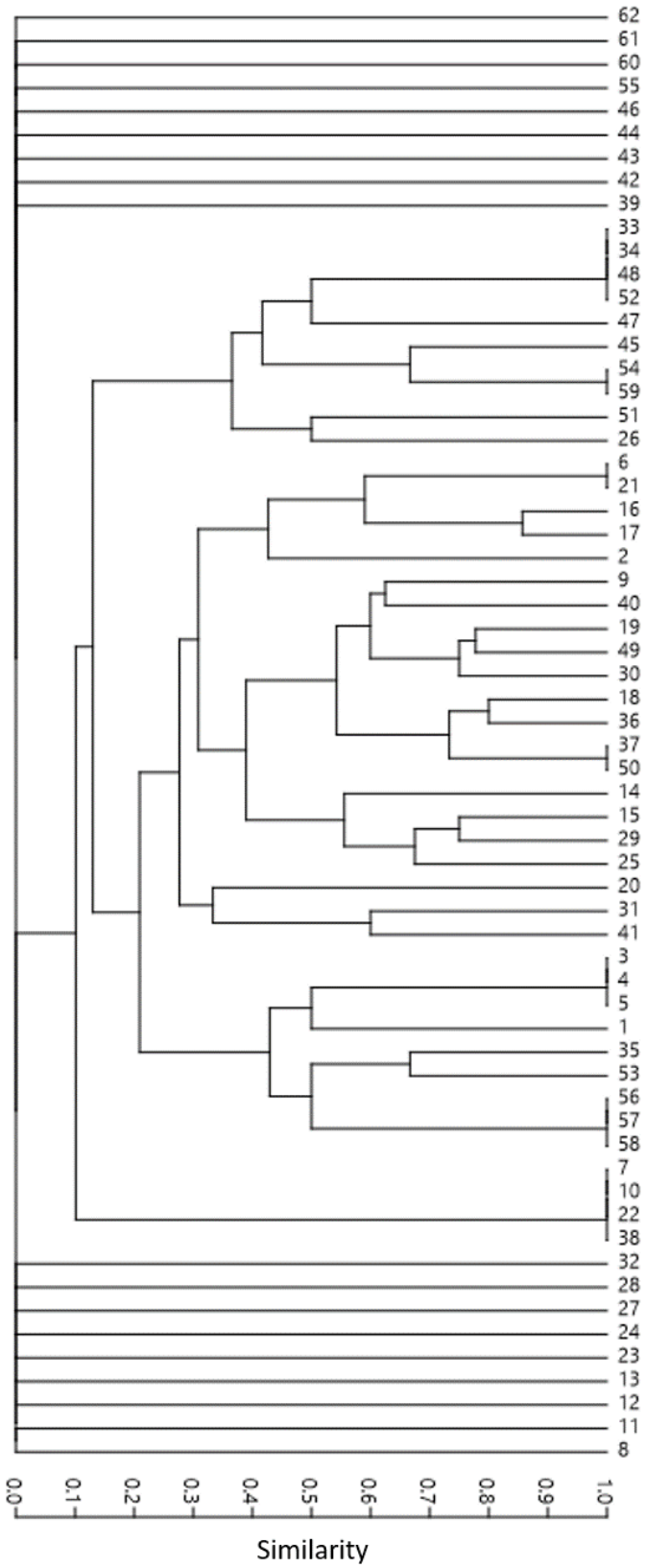

Figure 4│Dendrogram of the 62 potato accessions based on their Jaccard's similarity coefficients and UPGMA, using STM0031

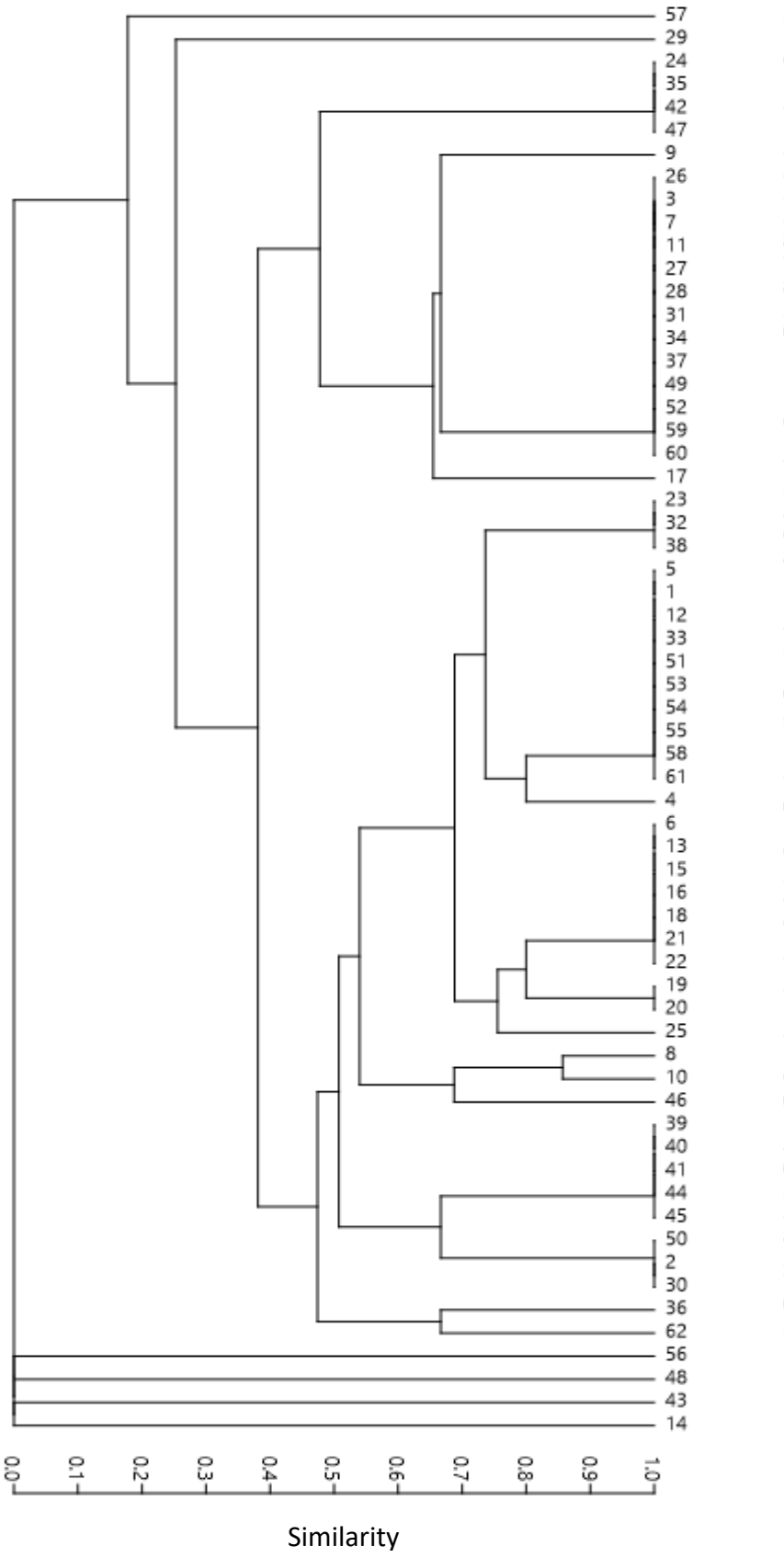

Figure 5│Dendrogram of the 62 potato accessions based on their Jaccard's similarity coefficients and UPGMA, using STM003

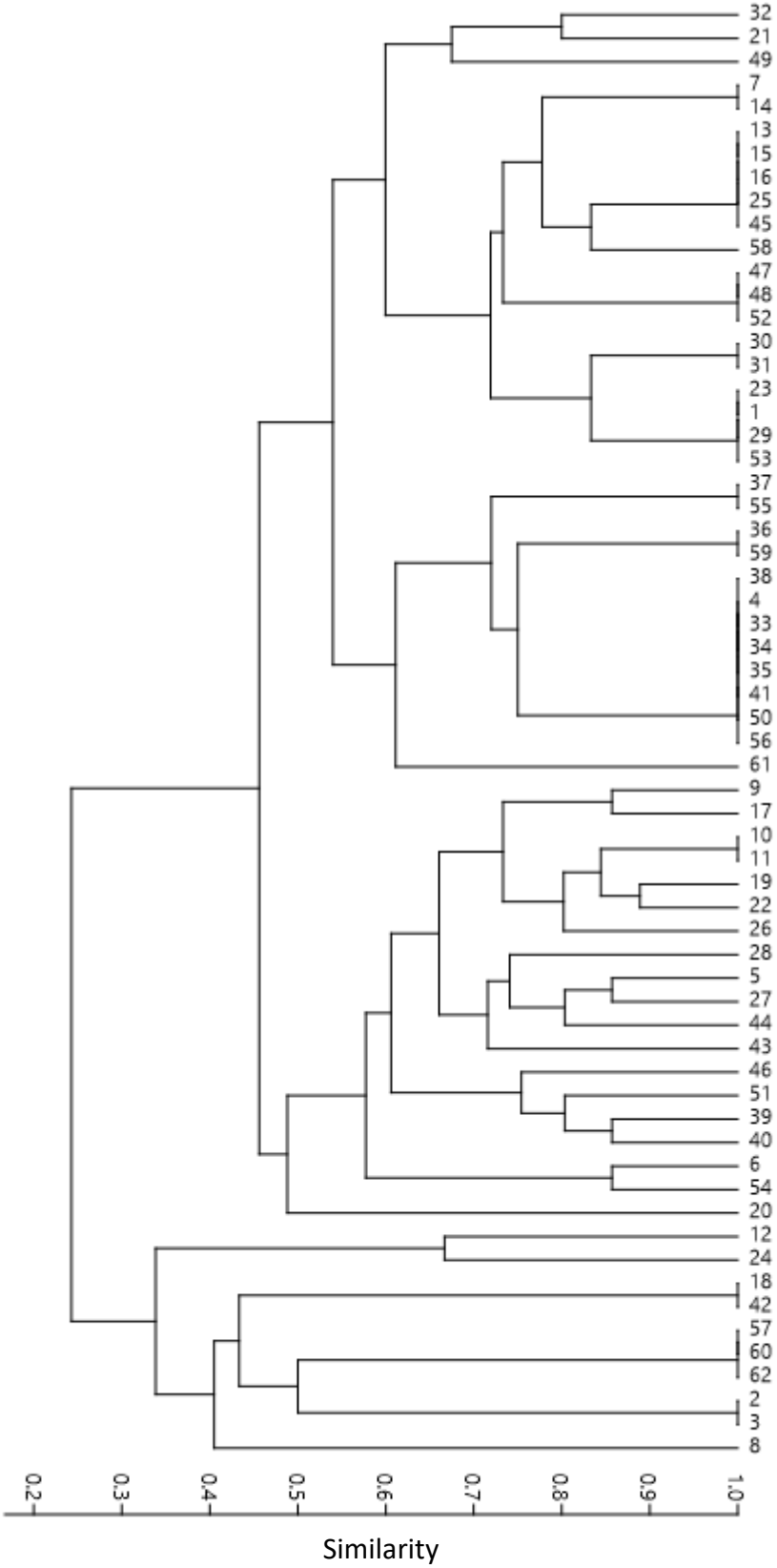

Figure 6 | Dendrogram of the 62 potato accessions based on their Jaccard's similarity coefficients and UPGMA, using STM1016

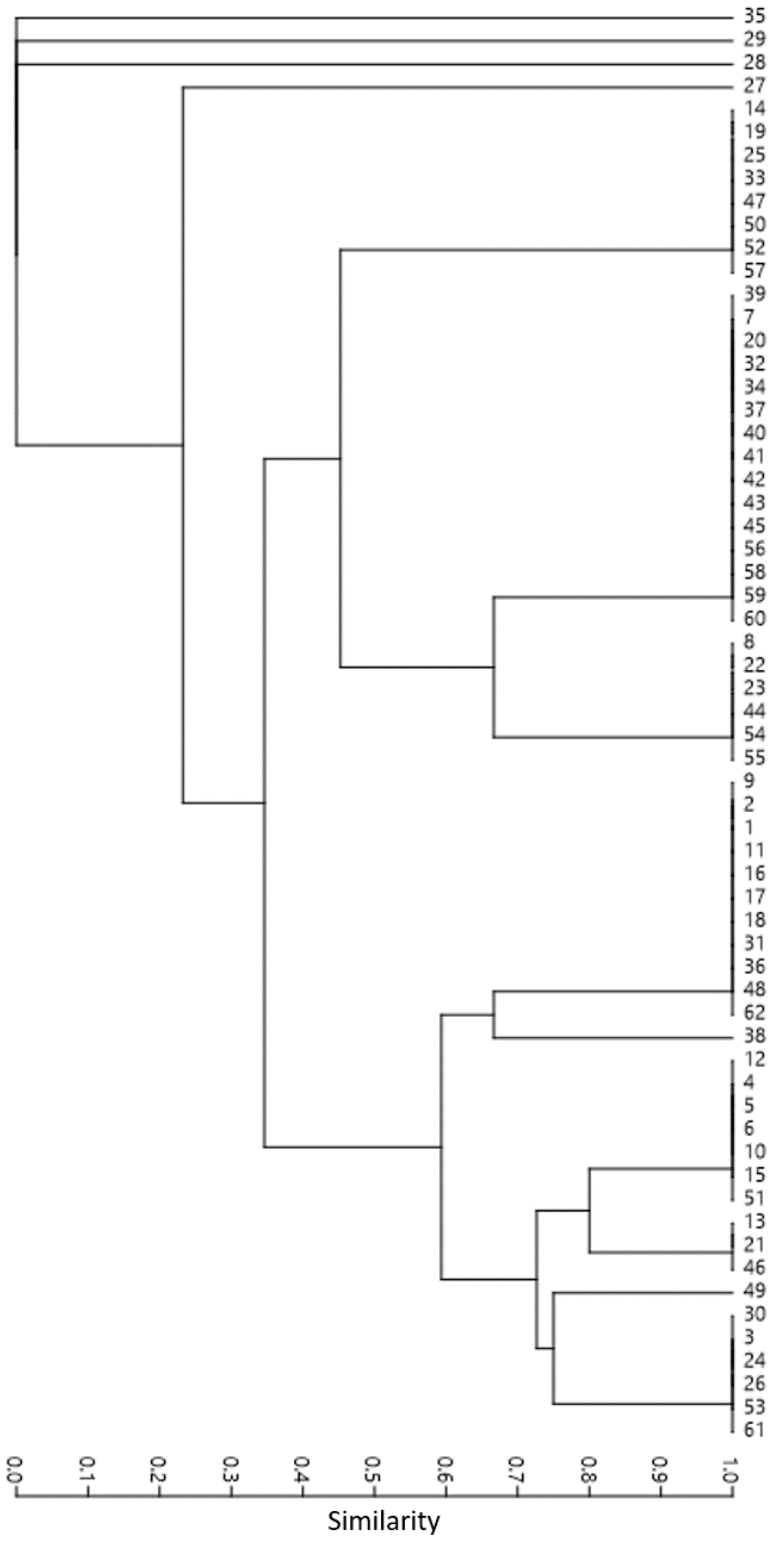

Figure 7| Dendrogram of the 62 potato accessions based on their Jaccard's similarity coefficients and UPGMA, using STM1049

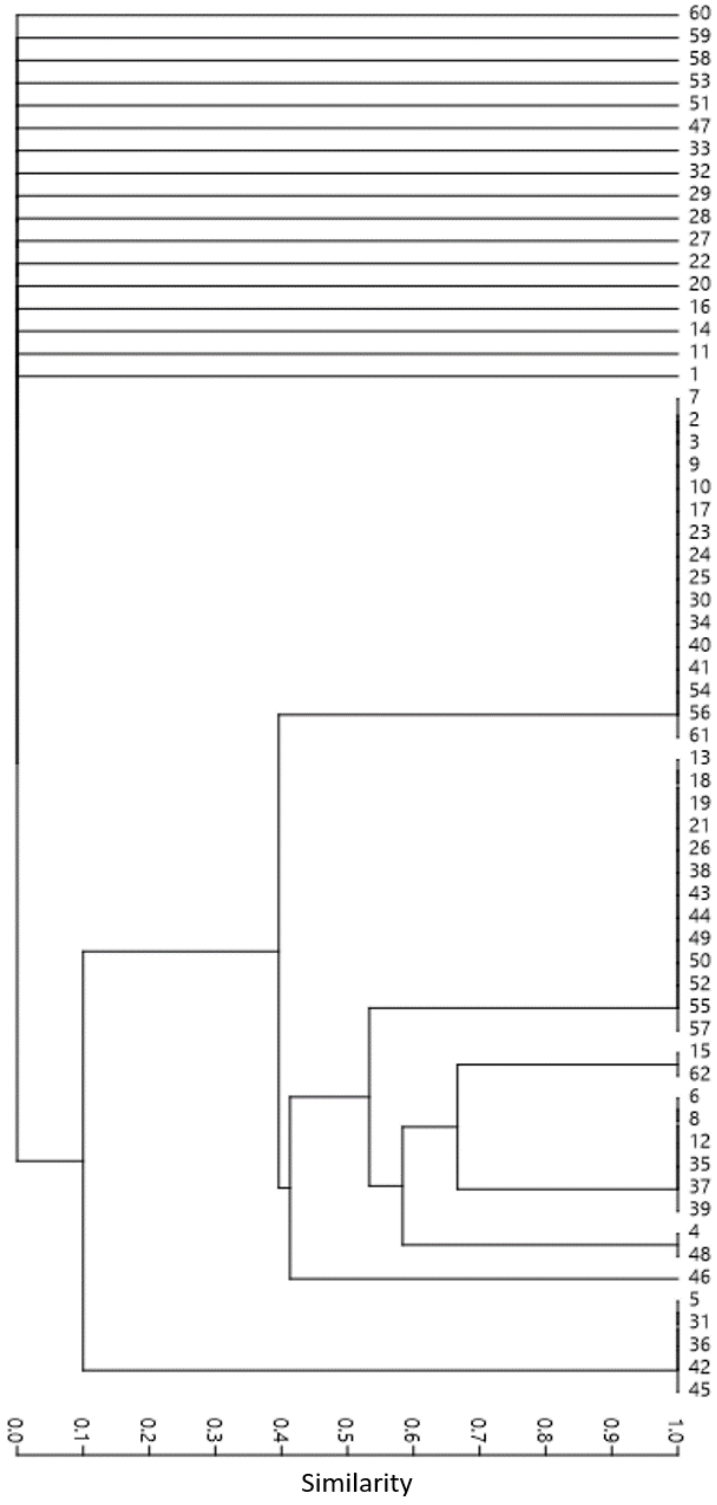

Figure 8| Dendrogram of the 62 potato accessions based on their Jaccard's similarity coefficients and UPGMA, using STM1052

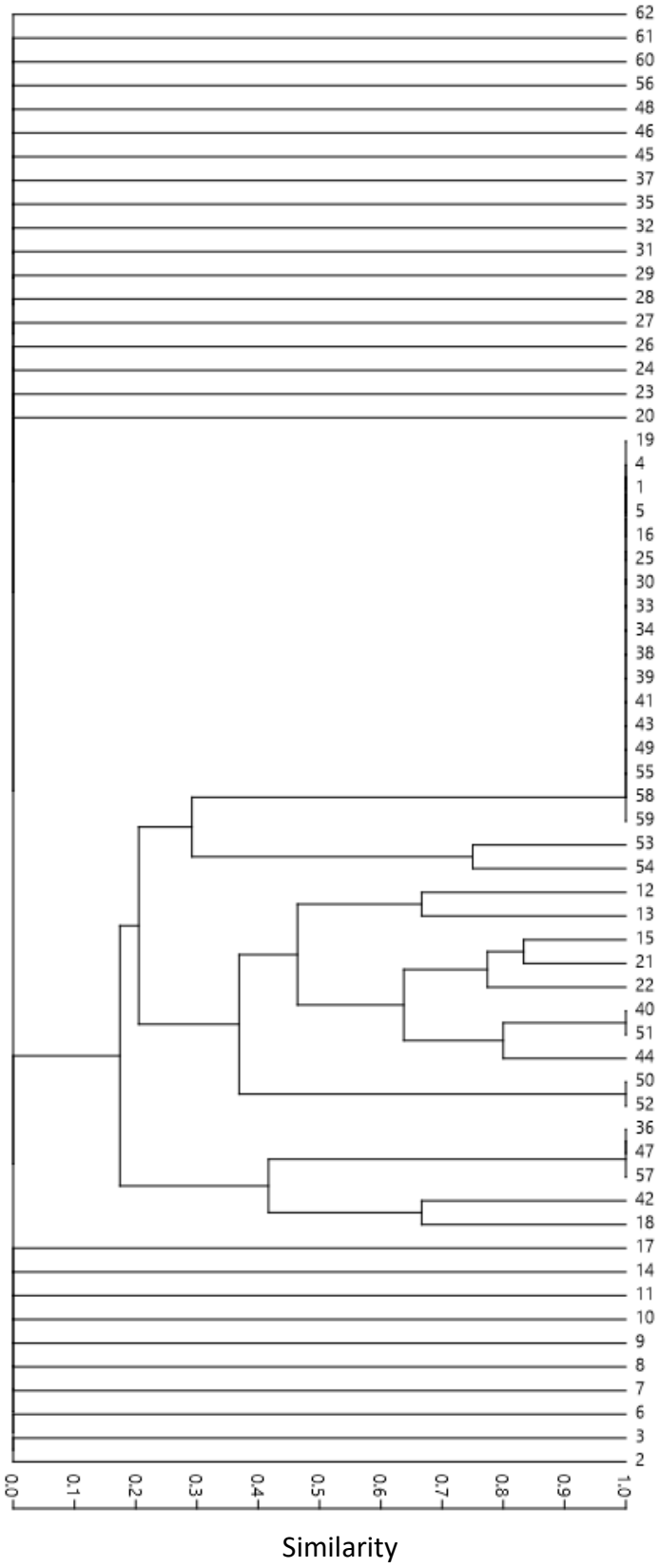

Figure 9│Dendrogram of the 62 potato accessions based on their Jaccard's similarity coefficients and UPGMA, using STM1106

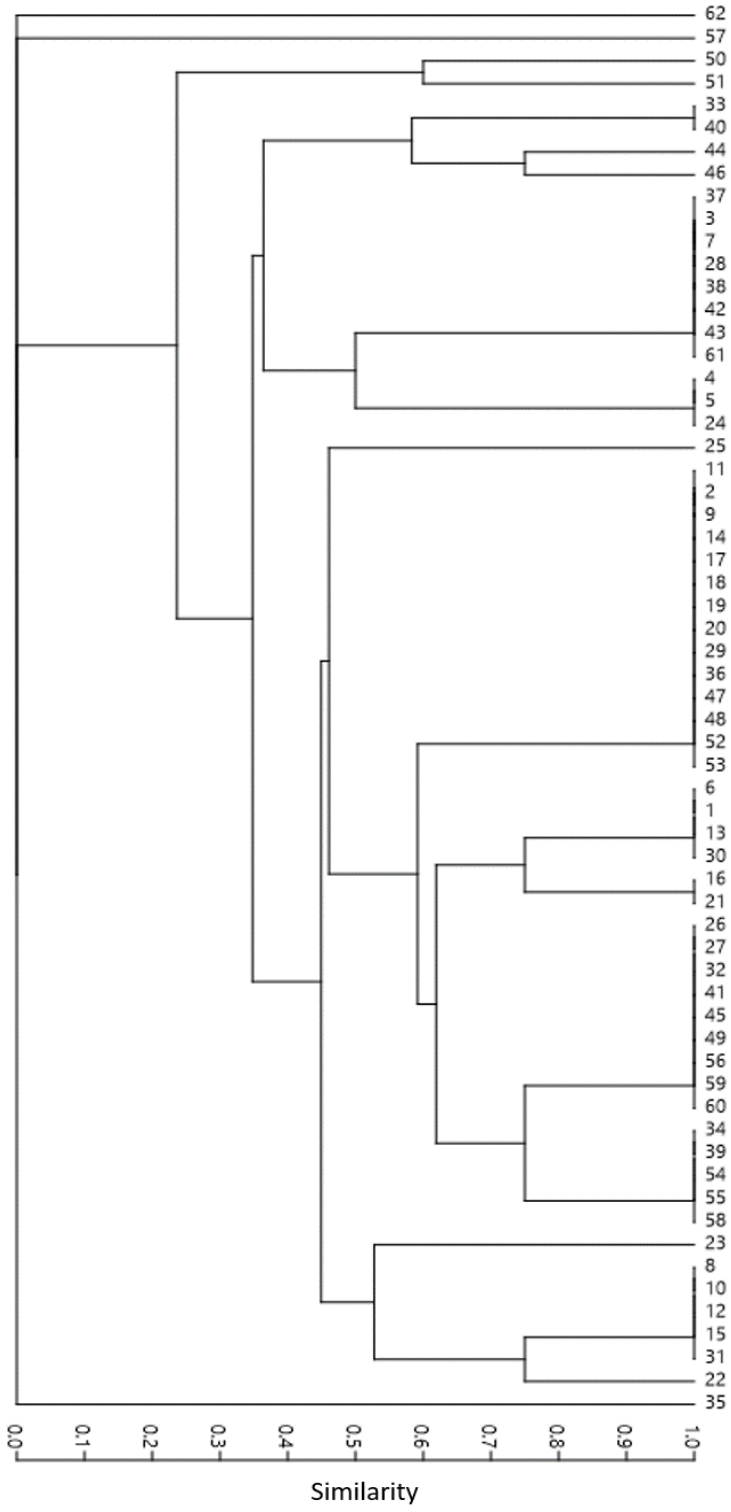

Figure 10| Dendrogram of the 62 potato accessions based on their Jaccard's similarity coefficients and UPGMA, using STM2013

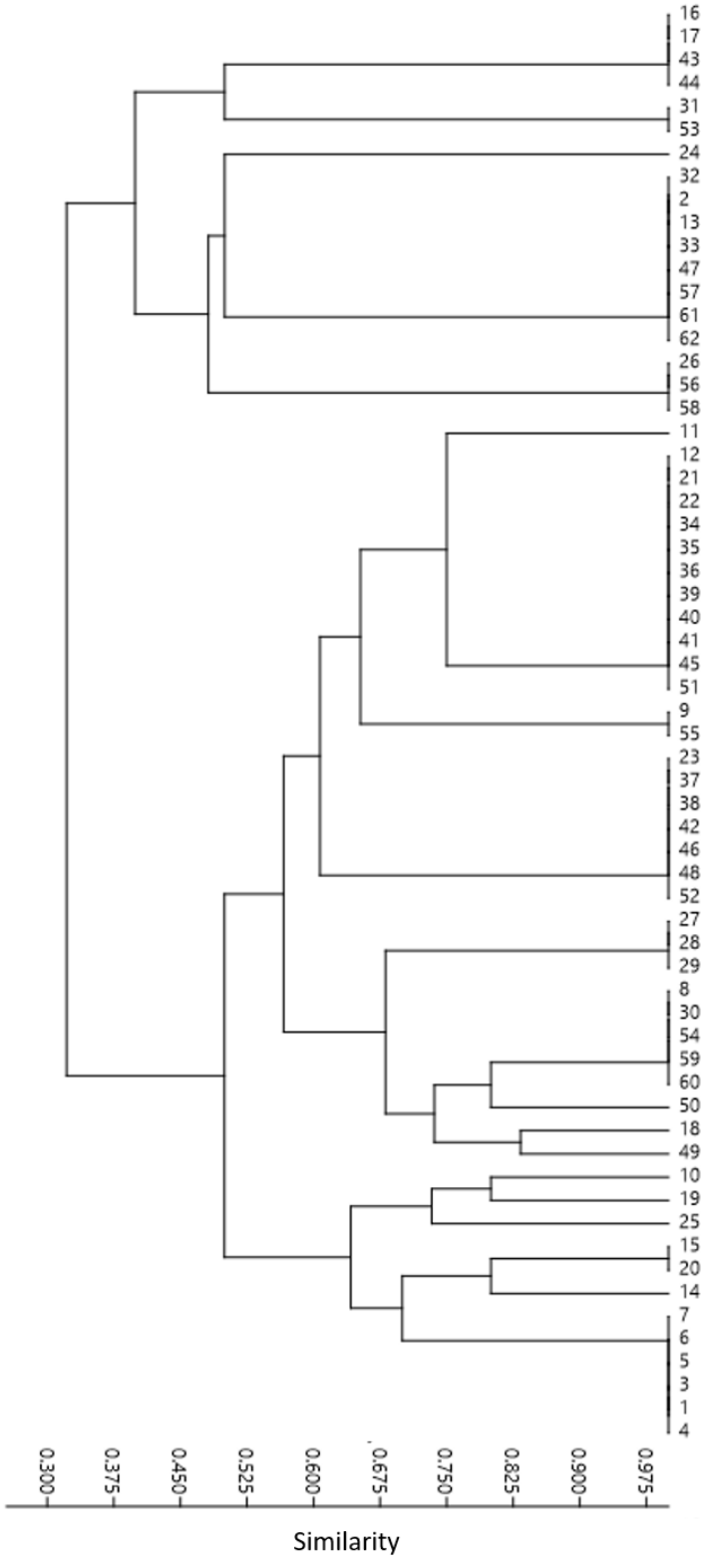

Figure 11 | Dendrogram of the 62 potato accessions based on their Jaccard's similarity coefficients and UPGMA, using STM3009

The rate of distinguishing cultivars by cluster (RDCC) was calculated (Table 5), using the dendrograms produced by each marker. The RDCC values show the ability of each SSR primer set to discriminate between the varieties, in other words a higher RDCC value would reflect a high discriminatory power. STM0031 and STM1106 gave the highest RDCC value, 67.74 and 61.29 respectively. Both the primers were able to distinguish about 40 cultivars by itself (i.e. 42 and 38 respectively). The lowest RDCC value, 9.68, was expressed by STM1049 which was able to distinguish only 9 cultivars out of the 62.

Table 7 | Ability of the 9 SSR markers to distinguish the potato cultivars.

SSR Marker	STM 0030	STM 0031	STM 0037	STM 1016	STM 1049	STM 1052	STM 1106	STM 2013	STM 3009
Ni	39	20	47	37	56	44	24	52	53
Nd	23	42	15	25	6	18	38	10	9
RDCC(%)	37.10	67.74	24.19	40.32	9.68	29.03	61.29	16.13	14.52

Ni, Number of indistinguishable cultivars; Nd, Number of distinguishable cultivars; RDCC: Rate of distinguishing cultivars by cluster

3.3 Genetic diversity analysis

To evaluate the genetic similarity among the 62 accessions form ACI Gene Bank, Jaccard's similarity coefficients were calculated, using the Past software (4.03). A frequency distribution curve, using Microsoft Excel, of the Jaccard’s similarity coefficients (Figure 2)

showed coefficients ranging from 0.182 to 0.778. The average value of obtained was 0.427 and the median value was 0.423, therefore the similarity coefficients among the accessions are suggested to be low. Hence, it is can be implied that there is a notable genetic diversity among the 62 varieties.

Only 24.9% of the accessions showed a similarity coefficient of more than 0.5 among which only 7 coefficient gave values higher than 0.7. Most of the similarity coefficient, 659, ranged from 0.400 to 0.488. 145 similarity coefficient expressed notably low values, between 0.182 and 0.297. The highest similarity coefficient was exhibited between accession 16 and accession 17 (ACI Alu 16 vs ACI Alu 17) giving a value of 0.778, while accessions 15 versus 22 (ACI Alu 15 vs ACI Alu 22) portrayed the second highest value, 0.771. Accession 57 (BARI Alu-08) vs accession 27 (ACI Alu 27) and accessions 57 ((BARI Alu-08) vs 11 (ACI Alu 6) both showed the lowest similarity coefficient, 0.182, in other words the two pairs showed the highest variation. One of the highest distances, 0.2, was also shown by accession 57 when paired with 54 (Bumrah).

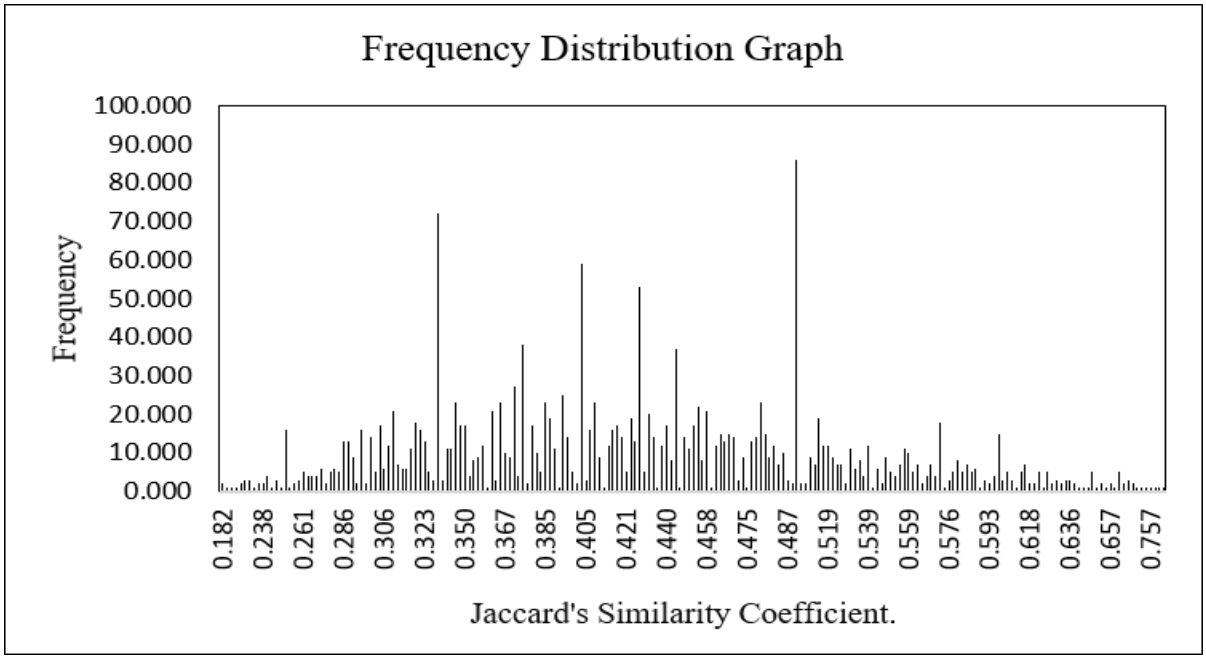

Figure 12 │ Frequency distribution curve of Jaccard's similarity coefficients, using 9 SSR markers, among the 62 accessions

Dendrogram generated by using UPGMA and Jaccard's similarity coefficient using the alleles produced by the amplified SSR markers showed the genetic relationship among the 62 accessions (Figure 3). The dendrogram generated 5 clusters: I, II, III, IV, and V (Table 5). Cluster V is composed of the majority of the accessions and is grouped into three sub groups, (i), (ii) and (iii). Sub group (i) consists of 35 accessions, with the highest genetic similarity coefficient being between accessions 16 and 17. Accession 46, Lavender, which is the only variety originated from Thailand (Table 2), showed an independent status in Cluster II. Accession 46 showed the highest genetic similarity (0.52) with accession 55 (BARI Alu-77), an UK origin cultivar, and the lowest genetic similarity (0.214 and 0.210) with accessions 24 and 43 (ACI Alu 24 and Sharposhona) respectively. The closest distance for accession 43 is 0.571 when paired with accession 42. Cluster I, II, III grouped a small number of accessions, each consisting 2, 1, 4 cultivars respectively. Cluster III, only consisting of 4 accessions, have the highest similarity coefficient between accession 24 and accession 43 (ACI Alu 24 and Sharposhona). The cophenetic relationship measured was 0.6457 ensuring the reliability of the dendrogram constructed using UPGMA and Jaccard's coefficient.

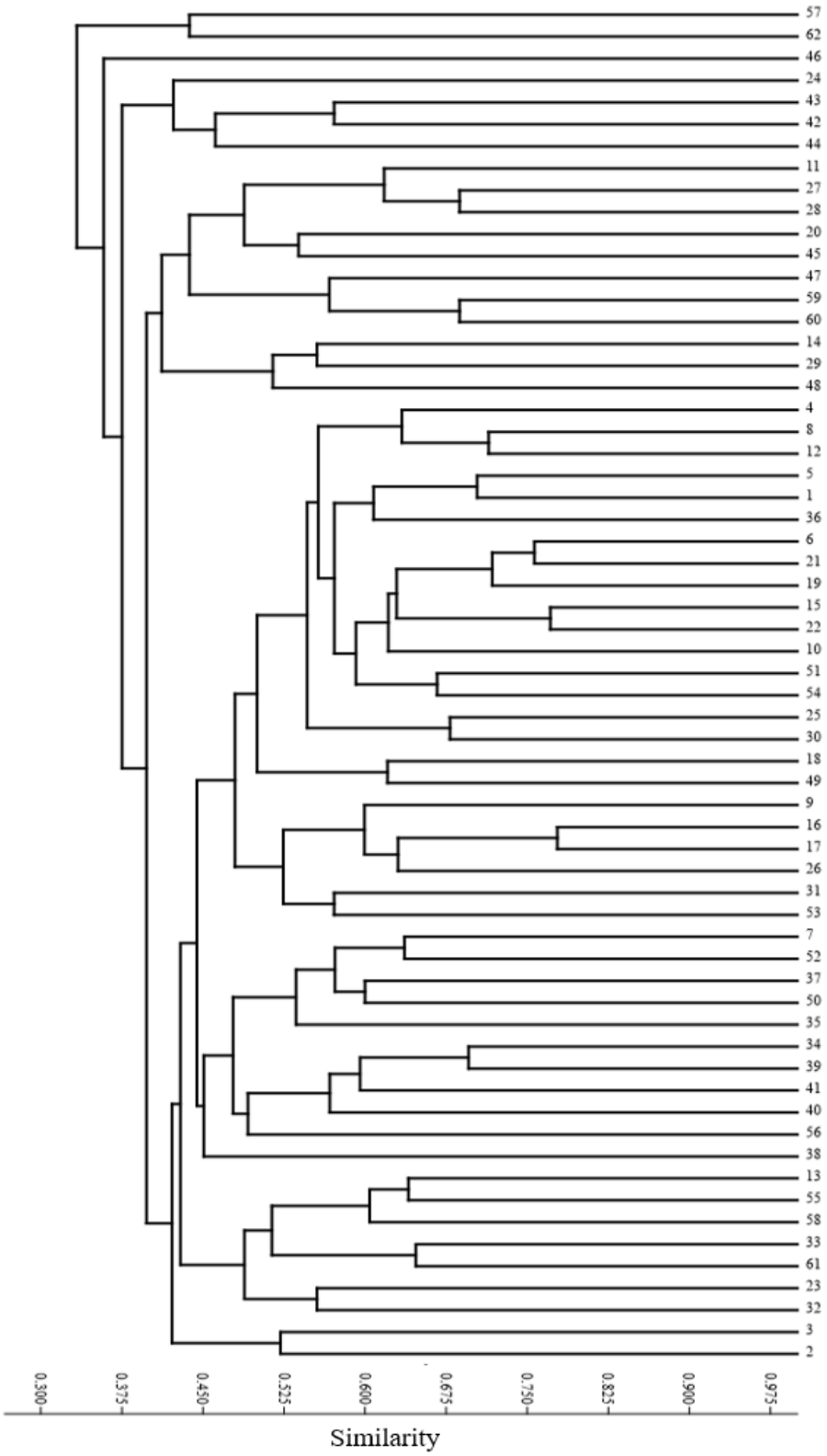

Figure 13│*Dendrogram of the 62 potato accessions based on their Jaccard's similarity coefficients and UPGMA, using all 9 SSR markers*

The dendogram constructed using a set of only the two best SSR markers, STM0031 and STM1016, according to their PIC values (Figure 14) was able to distinguish 56 of the 62 accessions. Meanwhile, four of the best primers in terms of their PIC values: STM0030, STM0031, STM0037 and STM1016 was able to reveal the genetic diversity of all the accessions (Figure 15).

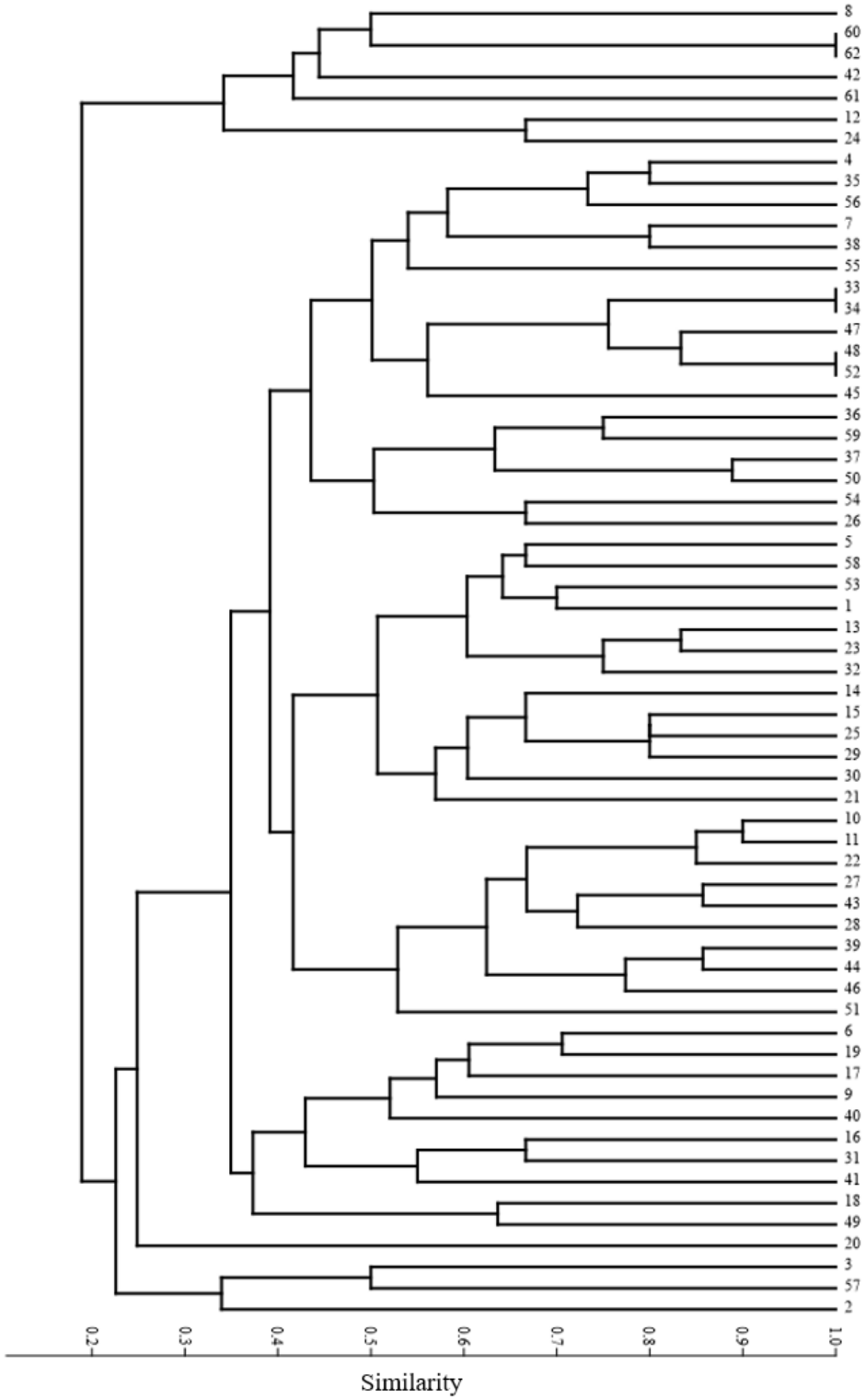

Figure 14│ *Dendrogram of the 62 potato accessions based on their Jaccard's similarity coefficients and UPGMA, using markers with the two highest PIC values: STM0031 and STM1016.*

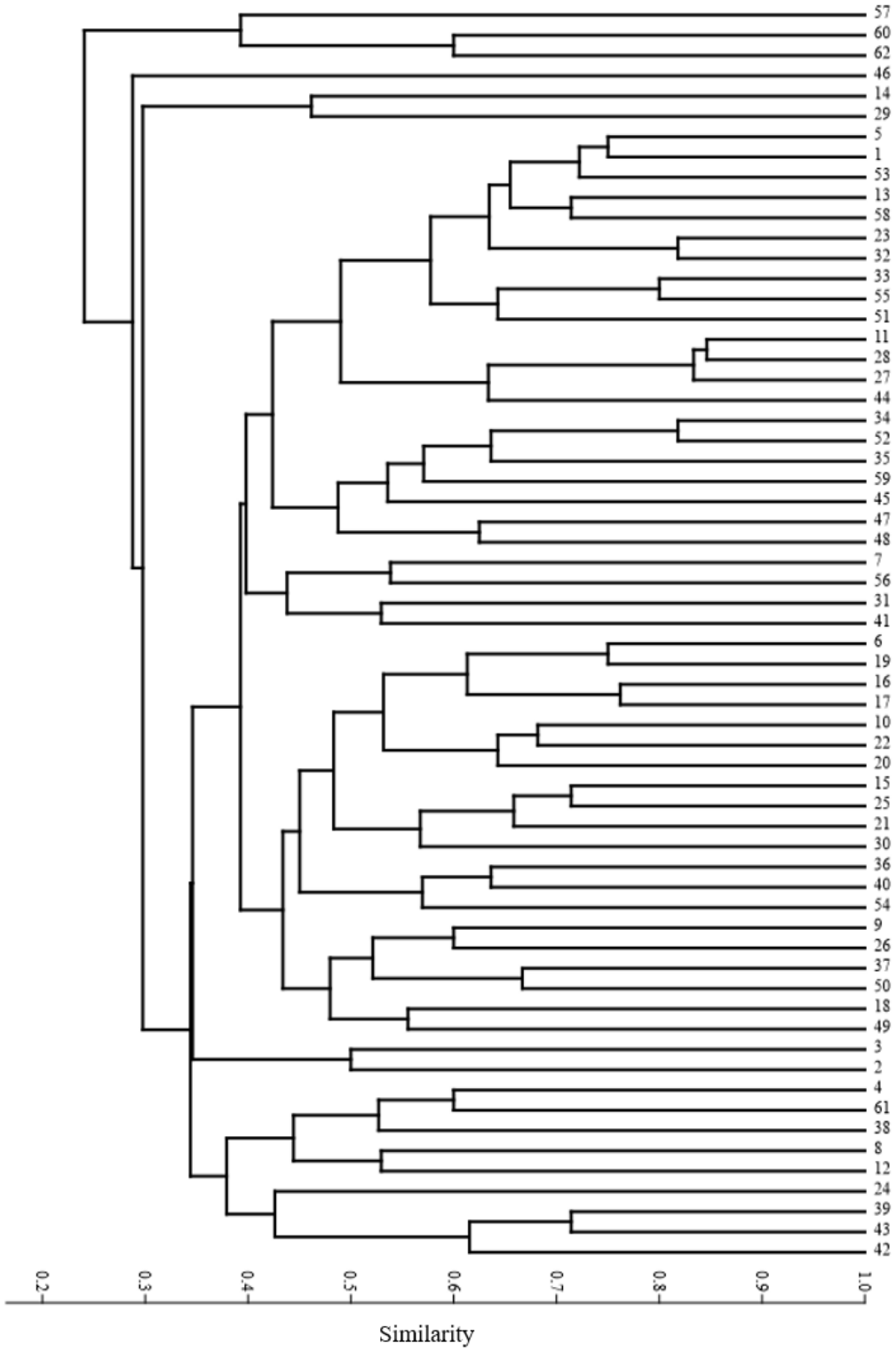

Figure 15│Dendrogram of the 62 potato accessions based on their Jaccard's similarity coefficients and UPGMA, using 4 markers with the highest PIC values: STM0030, STM0031, STM0037 and STM1016.

Chapter 4

[Discussion]

Potato is highly diverse in its characteristics in terms of its nutrition, shapes, sizes and colors (Sood et al., 2017). This study used 62 potato accessions from the ACI germplasm, with a range of phenotypic traits (Table 1) that are known to be beneficial agronomic and commercially viable. A few accessions with resistance to biotic stresses, which are major concerns for sustainable potato cultivation and breeding, were also included.

Ensuring the genetic diversity among potato cultivars, not just phenotypic, in germplasm banks is essential for efficient breeding programs. Information on the genetic relationship, using the allelic information per loci and genetic distances, among the varieties help in selection for the parent cultivar in designing breeding programs (Rahman *et al.,* 2022). This is because breeders need to avoid selecting closely related parental cultivars in order to avoid inbreeding and maintain potatoes with high heterozygosity (Demeke *et al.,* 1996; Mendoza and Haynes, 1974). This is required for commercial variety development such as fresh market use and French fries (Slater *et al.,* 2014).

The importance to safeguard the genetic diversity is underlined by FAO though the Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture (FAO 2012). It emphasizes on the conservation on plant genetic materials and their molecular and genomic information management in gene bank databases, with relevance to their morphological and agronomic traits. Moreover, The International Treaty on Plant Genetic Resources for Food and Agriculture highlights the conservation and sustainable use of plant genetic resources for food and agriculture (PGRFA) (FAO 2020). This treaty promotes the conservation and sustainable use of the PGRFA internationally and facilitates their exchange and sharing. Potato is one of the crops that is included in the international Treaty for Plant Genetic

Resources for Food and Agriculture (Arcimboldo, 2009). Hence, the importance of conservation of the diverse potato germplasm is reinforced in order to maintain both agriculture and food security.

There is also an effort to maintain databases of PGFA originating from gene banks of different countries. ASRBC, under ACI Limited, is one of the registered, under FAO, institutions which contributes to PGRFA (FAO 2023).

Hence, analysis on the genetic diversity was done on 62 germplasms of ACI gene bank using SSRs. SSRs are microsatellites that have shown to be reliable and precise molecular tools for genetic fingerprinting and thus have the ability to discriminate between potato varieties (Milbourne *et al.,* 1997; Powell *et al.*, 1996a).

As introduced before about microsatellites, the variation in allele number is due to difference in the number of repeat sequences that occur due to reasons such as slippage during replication (Provan et al., 1996) and mutation (Govindaraj, Vetriventhan and Srinivasan, 2015). And this variation or polymorphism is detected by SSR markers/primers.

The average allele per locus was 7.7 in this study and a similar value was obtained by Moisan-Thiery *et al.* (2005), 7.3; however, was notably higher than other studies such as Tillault and Yevtushenko (2019), 4.4; Fu *et al.* (2009), 6.4, and Moisen-Thiery *et al.* (2005), 6.6 alleles per locus. The number of alleles for STM0031 obtained from this study was 11, which was exactly the same with Ghislain *et al.* (2004) and other studies also showed a similar number, 10, by Ghislain *et al.* (2009) and Liao and Guo (2014). The lowest number of alleles was obtained from marker STM1052, 3 alleles, which was supported by Tillault and Yevtushenko (2019). However, this significantly differed from other studies such as Barandalla *et al.* (2006) and Ghislain *et al.* (2004, 2009) that detected much higher numbers of around 16 alleles for STM1052.

The PIC value, expressing the probability of an individual to be polymorphic at a particular locus, was the highest for STM0031 in our study i.e. 0888. This was relatively much higher compared to previous studies by Ghislain *et al.* (2008), Liao and Guo. (2014) and Tillault and Yevtushenko (2019) for ST0031; 0.721, 0.79 and 0.674 respectively. A similar high value was obtained for marker STM1016, 0.867, with the study from Ghislain *et al.* (2008), 0.840. The lowest PIC value, 0.621, was obtained by SSR marker STM 1052, similar to the values obtained by Tillault and Yevtushenko (2019) and Barandalla *et al.* (2006) where both studies obtained a PIC value of 0.64; meanwhile Bali et al (2017) detected an even lower PIC, 0.59. Although lower than Ghislain *et al.* (2004) and Liao and Guo (2014), 0.873 and 0.899 respectively; the PIC value obtained for STM2013 in this study was similar to that established by Barandalla *et al.* (2006), 0.76 and 0.73 respectively.

The comparison of PIC values and allele numbers obtained from various literature review have been evaluated in Table. Although the PIC value of STM 3009 was not obtained from literature review, it has been characterized and had been given a good quality score by Milbourne et al., 1998.

SSR marker	**STM 0030**	**STM 0031**	**STM 0037**	**STM 1016**	**STM 1049**	**STM 1052**	**STM 1106**	**STM 2013**	**STM 3009**
Tillault and Yevtushenko 2019	0.773 (6)	0.674 (4)	0.802	0.773		0.643	0.669		
Bali et al., 2016	0.55 (4)	0.67 (6)	0.00 (1)	0.75 (6)		0.59 (4)	0.58(4)		
Barandella et al., 2006	0.74 (5)		0.71 (6)		0.33 (2)	0.64 (3)	0.61 (3)	0.73 (5)	
Ghislain et al., 2004	0.864 (15)	0.771 (11)	0.787 (13)	0.776 (9)	0.771 (9)	0.832 (16)	0.822 (15)	0.873 (20)	
Ghislain et al., 2009	0.868 (19)	0.721 (10)	0.778 (17)	0.84 (17)	0.543 (9)	0.932 (17)	0.821 (17)		
Liao and Guo 2014	0.933 (18)		0.870 (14)		0.785 (7)		0.886 (14)	0.899 (12)	

Table 8 | PIC values (and Allele numbers in brackets) obtained from previous potato genetic diversity studies, using the SSR markers.

DNA fingerprinting of registered potato cultivars is essential for reasons including i) cultivar identification and verification (Ghislain, 2004; Milbourne *et al.*, 1997), ii) aiding cultivar selection in breeding programs (Milbourne et al., 1997) iii) conservation germplasm (Govindaraj et al., 2015; Liao and Guo, 2014). As such, the main objective of ACI Limited, is to maintain a gene bank with crops with high genetic diversity, subsequently this study analyzed the genetic diversity of selected 62 potato lines from the ACI gene bank. The varieties studied included most that originated from Bangladesh, and some that originated

from other countries such as the UK, Netherlands and Thailand which were acclimated to Bangladesh by ACI Ltd. In addition, potatoes with industrial purpose such as table potato, high yielding processing type, disease resistant potatoes were also tested. Despite the phenotypic differences of the varieties it is crucial to ensure both the genetic difference and relationship within each other to analyze the genetic diversity.

The Jaccard's similarity coefficient showed a high genetic diversity among the 62 accessions in our study. Only 24.9% of genotypes measured was more than 0.5 while the average value was 0.427, ensuring a high genetic diversity among the whole variety set in this study. In contrast, the potato varieties studied by Liao and Guo (2014), exhibited a lower genetic diversity where 99.70% of the genotypes showed a genetic similarity coefficient above 0.5987 with a mean being 0.6750. Our study showed a bimodal distribution, from the frequency curve obtained from using the Jaccard's similarity coefficient of all the genotypes and hence a better value of the central tendency of the similarity coefficients could be reflected by using the median value, 0.423. Other studies from Liao and Guo (2014), analyzing 85 potatoes, and Zhao *et al.* (2010), analyzing 30 rice varieties showed normal distribution in their frequency curve.

In this study we found the 62 accessions to have a Jaccard’s coefficient ranging from 0.182 to 0.778 and a similar difference in range was shown by (Favoretto et al., 2011), 0.41 to 0.93, with 38 potato accessions while using a similar number (10) of SSRs. Barandella *et a*l. (2006), also detected a similar range 0.57 to 1.00, with 41 varieties. Both the studies reported that their range corresponded to a high genetic variability among their potato varieties. Although the 20 potato varieties assessed by Tillault and Yevtushenko (2019), exhibited a larger difference in Jaccard's similarity coefficient range (0.080 to 0.824) the average obtained (0.397) was similar to our study.

In this study, Accession 46 (Lavender), the only variety in cluster II (Table 5), showed a low similarity coefficient with all other accessions; its highest value being only 0.52 with accession 55 (BARI Alu-77). Lavender, is also a table potato variety and in addition to the high genetic distance with most other varieties, it would deem Lavender an ideal parent cultivar for breeding. Accession 57, BARI Alu-08, originated from the Netherland and acclimatized in Bangladesh, is known to have properties for table and industrial purpose. Accessions 11, 16, 27, 31, 54 among other varieties have shown to be significantly genetically distinct from accession 57; 0.182, 0.233, 0.182, 0.222 and 0.2 respectively, hence any of these could be paired with BARI Alu-08 during breeding. In contrast, BARI Alu-08 grouped with accession 62 (BARI Alu-53), in cluster II, which also originated from the Netherlands and have table and industrial properties. Finally, accession 42 and accession 43 are varieties with highly beneficial traits, high yielding anti-oxidant rich and cyst nematode, PVY, PLRV resistant respectively. These two accessions could be of interest during parental selection as their genetic distances with most other cultivars are lower than 0.571.

The dendrogram constructed (Figure 13) showed a cophenetic correlation of 0.6457, ensuring its reliability since the minimum required value to ensure clustering consistency is 0.6 (Rocha *et al.*, 2010). An almost similar value was obtained, to our study, by Rocha *et al.* (2010), 0.68, when assessing the genetic diversity of 16 potatoes using Jaccard's coefficient and hence a well-founded representation of the similarity matrix was reported. The dendrogram showing the relationship of 22 cultivars by Tillault and Yevtushenko (2019) also gave a correlation coefficient value of 0.689.

A set of 4 of the best primers, according to their PIC values obtained in our study was able to discriminate among all the accessions (Figure 5). With a lower number of cultivars, 20, in the study done by Tillault and Yevtushenko (2019), combination of two primers sets were

reported to be able to distinguish among all the genotypes. It can be predicted that with a lower number of cultivars, a set of 2 primers may be used for future genetic diversity analysis.

Essentially, a genetic similarity lower than 0.95 between two cultivars, in at least 2 loci, will ensure the two cultivars being genetically different (Liao and Guo, 2014). However, using different sets of SSR primers can lead to a variation in clustering of the varieties (Liao and Guo, 2014). For instance, despite the similarity between a maternal line and its offspring, they may not be grouped in the same cluster on a Jaccard's similarity coefficient-based dendrogram (Tillault and Yevtushenko, 2019). This might be due to the high heterozygosity found in true seeds making them genetically distinct. Tillault and Yevtushenko (2019) suggested that adequate knowledge of the potato lineages must be known to draw a more accurate conclusion. Liao and Guo (2014) made a similar analysis regarding the necessity of information on potato origins due to the rise of new cultivar registration.

Moreover, Moisan-Thiery *et al.,* (2005) suggested that with an increase of new cultivars, analysis through DNA markers may result in identical molecular patterning among different potato cultivars. Hence, (i) genetically different potato varieties may end up in the same group of the constructed dendrogram, and (ii) this may lead to an inability of distinguishing between banding patterns of different varieties. As a result with the increase in novel cultivars, it is essential to screen and test for more marker systems (Moisan-Thiery *et al.,* 2005; Liao and Guo, 2014). A specific set of SSR markers should be constructed for a particular set of potatoes from a given origin, similar to the study done by Ghislain *et al.,* (2009). Furthermore, such sets of SSR markers could be developed that could be adapted to sequencing systems so as to analyze the diversity among a large sample of potatoes.

An area of interest to enhance research on is for the detection of varieties that are a result of recombinant technology or even is a result of random mutation (Moisan-Thiery *et al.*, 2005). Further research using SSR markers in genome edited mutation breeding experiments can progress the development of commercial potato lines. Screening of SSR markers that have association with target traits in addition to the identification of such genetically modified (GM) potatoes should be sought after to further accelerate the breeding process.

5 Conclusion

In this study a set of 9 SSR markers, with high discrimination power, were able to discriminate all the 62 potato varieties from ACI gene bank. The highest PIC obtained was from STM0031 and STM1016 with values of 0.888 and 0.867. An overall high genetic diversity is revealed by the Jaccard's coefficient following cluster analysis with the average genetic distance being 0.427. SSR marker STM0031 was able to distinguish the highest number of cultivars (42) and STM1049 the least (9). Overall, our findings can confirm with previous studies that SSR markers are easy to use, very informative and a reliable tool for molecular characterization of potato varieties. This study will serve as a confirmation of the 9 SSR markers to be able to distinguish and identify most cultivars which will ensure the maintenance of germplasm diversity. The information on genetic distances will also aid in desired trait selection which will ensure efficient and productive breeding process. Our future work will involve analyzing any association between the SSR markers and specific industrial potato traits, which may contribute in the identification of ACI developed GM potatoes and result in a more efficient potato production.

References

1. Acquaah, G. (2015), 'Conventional Plant Breeding Principles and Techniques', In Al-Khayri, J.M., Jain, S.M. and Johnson, D.V. (eds) *Advances in plant breeding*

strategies: breeding, biotechnology and molecular tools (No. 11474). Cham: Springer International Publishing. pp.115-158. doi: I 10.1007/978-3-319-22521-0_5

2. Bali, S., Sathuvalli, V., Brown, C., Novy, R., Ewing, L., Debons, J., Douches, D., Coombs, J., Navarre, D., Whitworth, J. and Charlton, B. (2017) 'Genetic fingerprinting of potato varieties from the northwest potato variety development program', *American Journal of Potato Research*, 94(1), pp. 54-63. doi: 10.1007/s12230-016-9547-z
3. Barandalla, L., de Galarreta, J.I., Rios, D. and Ritter, E. (2006) 'Molecular analysis of local potato cultivars from Tenerife Island using microsatellite markers. *Euphytica*, 152(2), pp.283-291. doi: 10.1007/s10681-006-9215-3
4. Benor, S., Zhang, M., Wang, Z. and Zhang, H. (2008) 'Assessment of genetic variation in tomato (Solanum lycopersicum L.) inbred lines using SSR molecular markers' *Journal of Genetics and Genomics*, 35(6), pp.373-379. doi: 10.1016/S1673-8527(08)60054-5
5. Bered, F., de Freitas Terra, T., Spellmeier, M. and Neto, J, F, B (2005) 'Genetic variation among and within sweet corn populations detected by RAPD and SSR markers' *Crop Breeding and Applied Biotechnology*, 5, pp.418-425
6. Bhardwaj, V., Salej, S., Ashwani, K., Vanishree, G., Sanjeev, S. and Sundaresha, S. (2019) 'Efficiency and reliability of marker assisted selection for resistance to major biotic stresses in potato' *Potato J*, *46*(1), pp.56-66.
7. Bradshaw, J.E. and Ramsay, G. (2005) 'Utilisation of the Commonwealth Potato Collection in potato breeding' *Euphytica*, 146, pp.9-19. doi: 10.1007/s10681-005-3881-4
8. Bradshaw, J.E., (2007) Potato-breeding strategy. In Dick Vreugdenhil, John Bradshaw, Christiane Gebhardt, Francine Govers, Donald K.L. Mackerron, Mark A.

Taylor, Heather A. Ross (eds) *Potato biology and biotechnology*, Elsevier Science B.V., pp.157-177

9. Brown, W.L. (1983) 'Genetic diversity and genetic vulnerability—an appraisal' *Economic botany*, *37*, pp.4-12. doi: 10.1007/BF02859301
10. Burgos, G., Zum Felde, T., Andre, C. and Kubow, S., (2020) 'The potato and its contribution to the human diet and health'. In H. Campos. and O. Ortiz., eds. *The Potato Crop Its Agricultural, Nutritional and Social Contribution to Humankind.* [online] Cham, Switzerland: Springer Nature, pp.37-74. Available at: https://doi.org/10.1007/978-3-030-28683-5 [Accessed on 1 September 2023].
11. Campos, H. and Ortiz, O. (2020). The potato crop: its agricultural, nutritional and social contribution to humankind. Cham, Switzerland: Springer Nature. p. 518
12. Carputo, D., Aversano, R. and Frusciante, L., 2004, June. Breeding potato for quality traits. In *Meeting of the Physiology Section of the European Association for Potato Research 684*, pp. 55-64. doi: 10.17660/ActaHortic.2005.684.7
13. Chimote, V.P., Chakrabarti, S.K., Pattanayak, D. and Naik, P.S. (2004) 'Semi-automated simple sequence repeat analysis reveals narrow genetic base in Indian potato cultivars' *Biologia plantarum*, 48, pp.517-522. doi: 0.1023/B:BIOP.0000047146.69936.ed
14. Coombs, J.J., Frank, L.M. and Douches, D.S. (2004). An applied fingerprinting system for cultivated potato using simple sequence repeats. *American Journal of Potato Research*, 81, pp.243-250. doi: 10.1007/BF02871765
15. Connell, K.H., (1951) 'The history of the potato' *The Economic History Review*, *3*(3), pp.388-395. doi.org/10.2307/2599996

16. de Haan. S. and Juárez, H. (2010) Land use and potato genetic resources in Huancavelica, central Peru, Journal of Land Use Science, 5:3, 179-195, doi: 10.1080/1747423X.2010.500681
17. Demeke, T., Lynch, D.R., Kawchuk, L.M., Kozub, G.C. and Armstrong, J.D. (1996) 'Genetic diversity of potato determined by random amplified polymorphic DNA analysis' *Plant Cell Reports,* 15, pp.662-667. doi: 10.1007/BF00231920
18. Devarumath, R.M., Kalwade, S.B., Kawar, P.G. and Sushir, K.V. (2012) 'Assessment of genetic diversity in sugarcane germplasm using ISSR and SSR markers' *Sugar Tech*, *14*, pp.334-344. doi: 10.1007/s12355-012-0168-7
19. Devaux, A., Kromann, P. and Ortiz, O. (2014) 'Potatoes for sustainable global food security' *Potato Research,* 57, pp.185-199. doi: 10.1007/s11540-014-9265-1
20. Devaux, A., Goffart, J.P., Kromann, P., Andrade-Piedra, J., Polar, V. and Hareau, G., (2021) 'The potato of the future: opportunities and challenges in sustainable agri-food systems' *Potato Research*, *64*(4), pp.681-720.
21. Devaux, A., Goffart, J.P., Petsakos, A., Kromann, P., Gatto, M., Okello, J., Suarez, V. and Hareau, G. (2020) 'Global food security, contributions from sustainable potato agri-food systems' *The Potato Crop: Its agricultural, nutritional and social contribution to humankind*. In H. Campos. and O. Ortiz., eds. *The Potato Crop Its Agricultural, Nutritional and Social Contribution to Humankind*. [online] Cham, Switzerland: Springer Nature, pp.3-35. Available at: https://doi.org/10.1007/978-3-030-28683-5 [Accessed 16 May 2023].
22. Douches, D.S., Maas, D., Jastrzebski, K. and Chase, R.W., (1996) 'Assessment of potato breeding progress in the USA over the last century' *Crop Science*, 36(6), pp.1544-1552. doi: 10.2135/cropsci1996.0011183X003600060024x

23. Duan, Y., Liu, J., Xu, J., Bian, C., Duan, S., Pang, W., Hu, J., Li, G. and Jin, L. (2019) 'DNA fingerprinting and genetic diversity analysis with simple sequence repeat markers of 217 potato cultivars (Solanum tuberosum L.) in China' *American journal of potato research*, 96, pp.21-32. doi: 10.1007/s12230-018-9685-6
24. Ellis, D., Salas, A., Chavez, O., Gomez, R., Anglin, N. (2020). Ex Situ Conservation of Potato [Solanum Section Petota (Solanaceae)] Genetic Resources in Genebanks. In H. Campos. and O. Ortiz. (eds) *The Potato Crop Its Agricultural, Nutritional and Social Contribution to Humankind*. [online] Cham, Switzerland: Springer Nature, pp.139–155. Available at: https://doi.org/10.1007/978-3-030-28683-5 [Accessed 16 May 2023].
25. FAO (2009) International year of the potato 2008: New light on a hidden treasure. https://www.fao.org/3/i0500e/i0500e00.htm Accessed 8 January 2023
26. FAO (2009b) International year of the potato 2008: New light on a hidden treasure. The Potato (Section 2) https://www.fao.org/3/i0500e/i0500e00.htm Accessed 8 January 2023
27. FAO (2012) Second Global Plan of Action For Plant and Genetic Resources for Food and Agriculture, Available at: https://www.fao.org/3/i2624e/i2624e00.pdf (Accessed on: 6 September 2023)
28. FAO (2020) High Level Political Forum 2020 Available at: https://sustainabledevelopment.un.org/content/documents/25926ITPGRFA_HLPF_2020_Inputs.pdf (Accessed on: 6 September 2023)
29. FAO (2023) *WIEWS* - World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture. Available at: https://www.fao.org/wiews/en/ (Accessed on: 7 September 2023)

30. Favoretto, P., Veasey, E.A. and Melo, P.C.T.D. (2011) 'Molecular characterization of potato cultivars using SSR markers' *Horticultura Brasileira*, 29, pp.542-547. doi: 10.1590/S0102-05362011000400017

31. Fowler, C. and Mooney, P.R (1990) 'Value of Diversity' In Fowler, C. and Mooney, P.R (eds) *Shattering: food, politics, and the loss of genetic diversity*. University of Arizona Press.

32. Fu, Y.B., Peterson, G.W., Richards, K.W., Tarn, T.R. and Percy, J.E. (2009) 'Genetic diversity of Canadian and exotic potato germplasm revealed by simple sequence repeat markers' *American Journal of Potato Research*, 86, pp.38-48. doi: 10.1007/s12230-008-9059-6

33. Gartner, U., Hein, I., Brown, L.H., Chen, X., Mantelin, S., Sharma, S.K., Dandurand, L.M., Kuhl, J.C., Jones, J.T., Bryan, G.J. and Blok, V.C. (2021) 'Resisting potato cyst nematodes with resistance' *Frontiers in Plant Science*, *12*, p.661194. doi: 10.3389/fpls.2021.661194

34. Gebhardt, C. (2013) 'Bridging the gap between genome analysis and precision breeding in potato' *Trends in Genetics*, 29(4), pp. 248-256. doi: 10.1016/j.tig.2012.11.006

35. Ghislain, M. and Douches, D.S. (2020). The Genes and Genomes of the Potato. In H. Campos. and O. Ortiz., eds. *The Potato Crop Its Agricultural, Nutritional and Social Contribution to Humankind*. [online] Cham, Switzerland: Springer Nature, pp. 139–155. Available at: https://doi.org/10.1007/978-3-030-28683-5 [Accessed 16 May 2023].

36. Ghislain, M., Núnez, J., del Rosario Herrera, M., Pignataro, J., Guzman, F., Bonierbale, M. and Spooner, D.M. (2009) 'Robust and highly informative

microsatellite-based genetic identity kit for potato' *Molecular breeding*, *23*, pp.377-388. doi:10.1007/s11032-008-9240-0

37. Ghislain, M., Spooner, D.M., Rodríguez, F., Villamón, F., Nunez, J., Vásquez, C., Waugh, R. and Bonierbale, M. (2004) 'Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato' *Theoretical and applied Genetics*, 108, pp.881-890. doi: 10.1007/s00122-003-1494-7
38. Govindaraj, M., Vetriventhan, M. and Srinivasan, M. (2015) 'Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives' *Genetics research international*, *2015.*
39. Giuseppe Arcimboldo (Food and Agriculture Organization of the United Nations), 2009, International Treaty on Plant Genetic Resources for Food and Agriculture Available at https://www.fao.org/3/i0510e/i0510e.pdf (Accessed: 4 September, 2023)
40. Hawkes, J.G., (1992) 'History of the potato'. In Harris, P.M. (ed) *The potato crop: The scientific basis for improvement*. London: Chapman & Hall, pp. 1-12.
41. Hasan, M., Seyis, F.A.T.İ.H., Badani, A.G., Pons-Kühnemann, J., Friedt, W., Lühs, W. and Snowdon, R.J. (2006) 'Analysis of genetic diversity in the Brassica napus L. gene pool using SSR markers' *Genetic Resources and Crop Evolution*, *53*, pp.793-802. doi: 10.1007/s10722-004-5541-2
42. Ispizua, V.N., Guma, I.R., Feingold, S. and Clausen, A.M. (2007) 'Genetic diversity of potato landraces from northwestern Argentina assessed with simple sequence repeats (SSRs)' Genetic Resources and Crop Evolution, *54*, pp.1833-1848. doi: 10.1007/s10722-007-9207-8

43. Jannat, A., Ishikawa-Ishiwata, Y. and Furuya, J. (2021) ‘Assessing the impacts of climate variations on the potato production in Bangladesh: A supply and demand model approach’ *Sustainability*, 13(9), p.5011. doi: 10.3390/su13095011

44. Jansky, S., Navarre, R. and Bamberg, J., (2019) ‘Introduction to the special issue on the nutritional value of potato’ *American Journal of Potato Research*, *96*, pp.95-97. doi.org/10.1007/s12230-018-09708-1

45. Jasim Aljumaili, S., Rafii, M.Y., Latif, M.A., Sakimin, S.Z., Arolu, I.W. and Miah, G. (2018) ‘Genetic diversity of aromatic rice germplasm revealed by SSR markers’ *BioMed research international*, *2018* doi: 10.1155/2018/7658032

46. Kalia, R.K., Rai, M.K., Kalia, S., Singh, R. and Dhawan, A.K. (2011) ‘Microsatellite markers: an overview of the recent progress in plants’ Euphytica, 177(3), pp.309-334. doi: 10.1007/s10681-010-0286-9

47. Kandemir, N., Yılmaz, G., Karan, Y.B. and Borazan, D. (2010) ‘Development of a simple sequence repeat (SSR) marker set to fingerprint local and modern potato varieties grown in central Anatolian Plateau in Turkey’ *African Journal of Biotechnology*, 9(34).

48. Kawchuk, L.M., Lynch, D.R., Thomas, J., Penner, B., Sillito, D. and Kulcsar, F., (1996) ‘Characterization of Solanum tuberosum simple sequence repeats and application to potato culiwar identification’ *American Potato Journal*, 73, pp.325-335. doi: 10.1007/BF02849164

49. Kolech, S.A., Halseth, D., Perry, K., De Jong, W., Tiruneh, F.M. and Wolfe, D. (2015) ‘Identification of farmer priorities in potato production through participatory variety selection’ *American journal of potato research*, *92*, pp.648-661. doi: 10.1007/s12230-015-9478-0

50. Lang, J., 2001. *Notes of a potato watcher* (No. 4). International Potato Center.

51. W. Legesse, B., A. Myburg, A., V. Pixley, K. and M. Botha, A. (2007) 'Genetic diversity of African maize inbred lines revealed by SSR markers' *Hereditas*, *144*(1), pp.10-17. doi: 10.1111/j.2006.0018-0661.01921.x

52. Liao, H. and Guo, H. (2014) 'Using SSR to evaluate the genetic diversity of potato cultivars from Yunnan province (SW China)' *Acta Biologica Cracoviensia s. Botanica*. doi: 10.2478/abcsb-2014-0003

53. Maldonado, L.A., Wright, J.E. and Scott, G.J. (1998) 'Constraints to production and use of potato in Asia' *American Journal of Potato Research*, *75*, pp.71-79. doi.org/10.1007/BF02883880

54. Manrique, L.A. (1993) 'Constraints for potato production in the tropics' *Journal of plant nutrition*, *16*(11), pp.2075-2120. doi: org/10.1080/01904169309364677

55. McGregor, C.E., Lambert, C.A., Greyling, M.M., Louw, J.H. and Warnich, L. (2000) A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (Solanum tuberosum L.) germplasm. *Euphytica*, *113*, pp.135-144. doi: /10.1023/A:1003925620546

56. Mendoza, H.A. and Haynes, F.L. (1974) 'Genetic basis of heterosis for yield in the autotetraploid potato' *Theoretical and applied Genetics*, 45(1), pp.21-25. doi: 10.1007/BF00281169

57. Milbourne, D., Meyer, R., Bradshaw, J.E., Baird, E., Bonar, N., Provan, J., Powell, W. and Waugh, R. (1997) 'Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato' *Molecular breeding*, 3, pp.127-136. doi: 10.1023/A:1009633005390

58. Milbourne, D., Meyer, R.C., Collins, A.J., Ramsay, L.D., Gebhardt, C. and Waugh, R., (1998). 'Isolation, characterisation and mapping of simple sequence repeat loci in

potato' *Molecular and General Genetics MGG*, 259, pp.233-245. doi: 10.1007/s004380050809

59. Moisan-Thiery, M., Marhadour, S., Kerlan, M.C., Dessenne, N., Perramant, M., Gokelaere, T. and Le Hingrat, Y. (2005) 'Potato cultivar identification using simple sequence repeats markers (SSR)' *Potato Research*, 48, pp.191-200. doi: 10.1007/BF02742376
60. Muthoni, J. and Nyamongo, D.O. (2009) 'A review of constraints to ware Irish potatoes production in Kenya' *Journal of horticulture and forestry*, *1*(7), pp.98-102. Available at http://www.academicjournals.org/jhf
61. Nei, M. (1973) 'Analysis of gene diversity in subdivided populations' *Proceedings of the national academy of sciences*, 70(12), pp.3321-3323. doi:10.1073/pnas.70.12.3321
62. Parida, S.K., Kalia, S.K., Kaul, S., Dalal, V., Hemaprabha, G., Selvi, A., Pandit, A., Singh, A., Gaikwad, K., Sharma, T.R. and Srivastava, P.S. (2009) 'Informative genomic microsatellite markers for efficient genotyping applications in sugarcane' *Theoretical and Applied Genetics*, *118*, pp.327-338. doi: 10.1007/s00122-008-0902-4
63. Powell, W., Machray, G.C. and Provan, J. (1996) 'Polymorphism revealed by simple sequence repeats' *Trends in plant science*, 1(7), pp.215-222. doi: 10.1016/1360-1385(96)86898-1
64. Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A. (1996) 'The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis' *Molecular breeding*, 2, pp.225-238. doi: 10.1007/BF00564200

65. Provan, J., Powell, W. and Waugh, R. (1996) 'Microsatellite analysis of relationships within cultivated potato (Solanum tuberosum)' *Theoretical and Applied Genetics*, 92(8), pp.1078-1084. doi: 10.1007/BF00224052

66. Raihan, A., Nahiyan, A., Rahman, A. and Rahman, L. (2016) 'Molecular characterization of selected M5 lines of rice after TILLING for salinity tolerance using 20 SSR primers' *British Biotechnology Journal*, 12(4), pp.1-11. doi:10.9734/BBJ/2016/23868

67. Rahman, S.U., Jamil, S., Shahzad, R., Yasmeen, E., Sattar, S. and Iqbal, M.Z. (2022) 'Genetic diversity and DNA fingerprinting of potato varieties using simple sequence repeat (SSR) markers' *Journal of Animal & Plant Sciences*, 32(3), pp.775-783. doi: 10.36899/JAPS.2022.3.0479

68. Reddy, B.J., Mandal, R., Chakroborty, M., Hijam, L. and Dutta, P. (2018) 'A review on potato (Solanum tuberosum L.) and its genetic diversity' *International Journal of Genetics*, 10(2), pp.360-364. doi: 10.9735/0975-2862.10.2.360-364

69. Rocha, E.A., Paiva, L.V., Carvalho, H.H.D. and Guimarães, C.T. (2010) 'Molecular characterization and genetic diversity of potato cultivars using SSR and RAPD markers' *Crop Breeding and Applied Biotechnology*, 10, pp.204-210. doi: 10.1590/S1984-70332010000300004

70. Roy, T.S., Chakraborty, R., Parvez, M.N., Biswas, S. and Chakraborty, S., (2017) 'Development of sustainable gross national income from potato export in Bangladesh-A perspective review' *Universal Journal of Agricultural Research*, *5*(1), pp.46-51. doi: 10.13189/ujar.2017.050107

71. Rupp, J.V., Mangolin, C.A., Scapim, C.A. and Machado, M.D.S. (2009) 'Genetic structure and diversity among sweet corn (su1-germplasm) progenies using SSR marker' *Maydica*, *54*(1), pp.125-132.

72. Scott, G.J. and Suarez, V., 2012. The rise of Asia as the centre of global potato production and some implications for industry. *Potato Journal, 39(1),* pp.1-22

73. Singh, N., Choudhury, D.R., Tiwari, G., Singh, A.K., Kumar, S., Srinivasan, K., Tyagi, R.K., Sharma, A.D., Singh, N.K. and Singh, R. (2016) 'Genetic diversity trend in Indian rice varieties: an analysis using SSR markers' *BMC genetics*, *17*, pp.1-13. doi: 10.1186/s12863-016-0437-7

74. Sharma, V. and Nandineni, M.R. (2014) 'Assessment of genetic diversity among Indian potato (Solanum tuberosum L.) collection using microsatellite and retrotransposon based marker systems' *Molecular phylogenetics and evolution*, *73*, pp.10-17. doi: 10.1016/j.ympev.2014.01.003

75. Slater, A.T., Cogan, N.O., Hayes, B.J., Schultz, L., Dale, M.F.B., Bryan, G.J. and Forster, J.W. (2014) 'Improving breeding efficiency in potato using molecular and quantitative genetics' *Theoretical and applied genetics*, 127, pp.2279-2292. doi: 10.1007/s00122-014-2386-8

76. Sood, S., Bhardwaj, V., Pandey, S.K., Chakrabarti, S.K. (2017) 'History of Potato Breeding: Improvement, Diversification, and Diversity' In: Kumar Chakrabarti, S., Xie, C., Kumar Tiwari, J. (eds) The Potato Genome. Compendium of Plant Genomes. Springer, Cham. doi: 10.1007/978-3-319-66135-3_3

77. Tang, R., Gao, G., He, L., Han, Z., Shan, S., Zhong, R., Zhou, C., Jiang, J., Li, Y. and Zhuang, W. (2007) 'Genetic diversity in cultivated groundnut based on SSR markers' *Journal of Genetics and Genomics*, *34*(5), pp.449-459.

78. Taylor, A.S. and Dawson, P. (2021) 'Major constraints to potato production in Indonesia: A review' *American Journal of Potato Research*, *98*(3), pp.171-186. doi:10.1007/s12230-021-09831-6

79. Tillault, A.S. and Yevtushenko, D.P. (2019) ‘Simple sequence repeat analysis of new potato varieties developed in Alberta, Canada’ *Plant Direct*, 3(6), p.e00140. doi: 10.1002/pld3.140

80. Tiwari, J.K., Chandel, P., Gupta, S., Gopal, J., Singh, B.P. and Bhardwaj, V. (2013) ‘Analysis of genetic stability of in vitro propagated potato microtubers using DNA markers’ *Physiology and Molecular Biology of Plants*, 19, pp.587-595. doi: 10.1007/s12298-013-0190-6

81. Uddin, A.S., Rahman, M.M., Kamaly, M.H.K., Alam, M.B. and Sheikh, M.M. (2015) 'Constraints and suggestions for modern variety potato production technology’ *Bangladesh Journal of Agricultural Research*, 40(1), pp.95-108. doi: 10.3329/bjar.v40i1.23763

82. Vieira, M.L.C., Santini, L., Diniz, A.L. and Munhoz, C.D.F. (2016). ‘Microsatellite markers: what they mean and why they are so useful’, *Genetics and Molecular Biology*, 39(3), pp.312-328. doi: 10.1590/1678-4685-GMB-2016-0027

83. Wang, L., Guan, R., Zhangxiong, L., Chang, R. and Qiu, L. (2006) ‘Genetic diversity of Chinese cultivated soybean revealed by SSR markers’ *Crop Science*, *46*(3), pp.1032-1038. doi: 10.2135/cropsci2005.0051

84. Wang, Y., Rashid, M.A.R., Li, X., Yao, C., Lu, L., Bai, J., Li, Y., Xu, N., Yang, Q., Zhang, L. and Bryan, G.J. (2019) ‘Collection and evaluation of genetic diversity and population structure of potato landraces and varieties in China’ *Frontiers in Plant Science*, 10, pp.139. doi: 10.3389/fpls.2019.00139

85. Wang, Z., Weber, J.L., Zhong, G. and Tanksley, S.D. (1994) ‘Survey of plant short tandem DNA repeats’ *Theoretical and applied genetics*, 88, pp.1-6. doi: org/10.1007/BF00222386

86. Wu, W., Yu, Q., You, L., Chen, K., Tang, H. and Liu, J. (2018) 'Global cropping intensity gaps: Increasing food production without cropland expansion' Land use policy, *76*, pp.515-525.

87. Zhao, Q.Y., Zhang, Y.D. and Wang, C.L., (2010) 'Analysis on genetic diversity of 30 Japonica rice varieties using SSR markers' *Journal of Plant Genetic Resources*, 11(2), pp.218-223.