Title: Potato Variety Improvement through Marker Assisted Selection

By

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Master of Science in Biotechnology

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Declaration

It is hereby declared that

1. The thesis submitted is my 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 have acknowledged all main sources of help.

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Approval

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Abstract

Late blight (LB), caused by *Phytophthora infestans* (Mont.) de Bary, is the most destructive for potato. The main objective of this study was to evaluate 72 potato lines, derived from cross products of the recipient cv. ACI Pakri-1 and a LB resistant donor variety, against late blight. The experiment was conducted with three levels of inoculums pressure (i) LB inoculation & no fungicide, (ii) No LB inoculation & no fungicide and (iii) No LB inoculation & fungicide. LB infection was assessed at 10 days interval by scoring the percentage of foliage destruction. Three categories of potato lines were selected considering level of LB infection and tuber appearance - (a) LB resistant - 8 lines, (b) LB tolerant - 14 lines, and (c) LB susceptible - 14 lines. The foliage destruction of selected LB resistant lines was considered to be between 1 to 25%, in LB tolerant lines between 25 to 50 %, and in LB susceptible lines between 51 to 90% at 85 DAP. The recipient ACI Pakri-1 had 100% foliage destruction at 63-65 DAP. On the other hand, lowest foliage destruction 1-2% was recorded in Line 8. In LB resistant lines low AUDPC, rAUDPC and susceptible scale value were found. The highest susceptible scale value 9 was recorded mostly in LB susceptible cv. ACI Pakri-1 and BARI Alu-40; where the range of susceptible scale value 0.1 was calculated in line. However, LB resistant gene Rpi-abpt and Rpi-blb1, amplified using marker R2-F1/R2-R3 and 1521/518 respectively, were identified through marker assisted selection (MAS) in the donor variety and crossed LB resistant line 8, 13, 41, 61, 72 and 54 but absent in recipient ACI Pakri-1. Round tubers, mostly of uniform size, deep to light red and white skin, red and deep eye were found in resistant lines. The highest number of tubers/ hill (20) was recorded in line 8 under natural inoculums pressure, and the highest weight of tuber/hill (460g) was found in line 8 and yield was 38.3 Mt/ha, which was higher than the donor variety (31.5 Mt/ha) and the recipient ACI Pakri-1 (29.0 Mt/ha) under fungicide application treatment. **Finally, based on the potentiality against LB and yield performance, Line 8 was registered as potato variety namely ACI LBR Pakri-1 by Seed Wing, Ministry of Agriculture, Bangladesh.**

Keywords: Potato, LB resistance, inoculums pressure, AUDPC, rAUDPC, susceptible scale value, MAS

Dedicated

To my

Beloved Parents and my

Loving Wife

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It's my honor to write this acknowledgement to express my gratefulness to people who have helped me throughout this research work.

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List of Acronym

- LATE BLIGHT - LB
- DAYS AFTER PLANTATION - DAP
- AREA UNDER DISEASE PROGRESSION CURVE - AUDPC
- RELATIVE AREA UNDER DISEASE PROGRESSION CURVE - rAUDPC
- TRUE POTATO SEED - TPS
- TUBER CROP RESEARCH CENTRE - TCRC
- HIGH YIELDING VARIETIES - HYV
- INTERNATIONAL POTATO CENTRE - CIP
- BANGLADESH AGRICULTURAL RESEARCH INSTITUTE - BARI
- BANGLADESH BUREAU OF STATISTICS - BBS
- BANGLADESH AGRICULTURAL DEVELOP MENT CORPORATION- BADC
- INDIGENOUS POTATO VARIETY - IPV
- MODERN POTATO VARIETY - MPV
- EFFECTOR TRIGGERED IMMUNITY - ETI
- HYPERSENSITIVE RESPONSE - HR
- SIMPLE SEQUENCE REPEAT - SSR
- TISSUE CULTURE - TC
- MARKER ASSISTED SELECTION - MAS
- DEOXYRIBONUCLEIC ACID - DNA

- GIBERELLIC ACID - GA3
- CETYLTRIMETHYLAMMONIUM BROMIDE - CTAB
- POLYMERASE CHAIN REACTION - PCR
- ETHYLENEDIAMINETETRAACETIC ACID - EDTA
- ULTA VIOLET - UV

Chapter 1

1. Introduction

Potato (*Solanum tuberosum* L.) is a vegetable crop belongs to the nightshade (Solanaceae) family. Till date, 110 wild and 4 cultivated species of potato has been recorded and It is highly heterozygous in nature with a ploidy level ranging from diploid to hexaploidy (Slater et al., 2014). However, the cultivated potatoes are tetraploid (2n=4x=48). It is a short-lived perennial plant that is cultivated as an annual crop. The economically significant part of this plant is the modified stems called 'tubers' grown under the soil along with stolon where large amount of carbohydrate is stored. Though potato plants can produce seeds in their berries, known as true botanical seeds or true potato seeds (TPS), the tubers are the primary propagules (Bradeen and Haynes, 2011). Potato plants are herbaceous perennials that grow about 60 cm (24 in) high, counting on variety, with the leaves dying back after flowering, fruiting and tuber formation. They bear white, pink, red, blue, or purple flowers with yellow stamens. Generally, the tubers of sorts with white flowers have white skins, while those of sorts with colored flowers tend to possess pinkish skins (Winch, 2006). Potatoes are mostly cross-pollinated by insects like bumblebees, which carry pollen from other potato plants, although a considerable amount of self-fertilizing occurs as well. Tubers form in response to decreasing day length, although this tendency has been minimized in commercial varieties (iKISAN).

After flowering, potato plants produce small green fruits that resemble green cherry tomatoes, each containing about 300 seeds. Like all parts of the plant except the tubers, the fruit contain the toxic alkaloid solanine and are therefore unsuitable for consumption. New varieties grown from TPS are often propagated vegetatively by planting tubers, pieces of tubers move include a minimum of one or two eyes, or cuttings, a practice utilized in greenhouses for the assembly of healthy seed tubers.

Plants propagated from tubers are clones of the parent, whereas those obtained from TPS produce a wide range of variation.

Fig.1. Flowering of potato plants & whole potato plants

The root of potato is adventitious arising from the base of a sprout. Root growth is usually restricted to top layers at a depth 20 - 25 cm. In rich soils roots of some varieties may reach up to 90 - 100 cm. Such deep roots are found in potatoes make an exception in dicotyledonous plants, which have tap root system.

The stem of potato is stout, erect, flexuous, much branched, 30 - 60 cm long, slightly hairy, distinctly winged on the angles. The upper most part of the sprout develops into aerial stem or haulms. The aerial stems are angular, pubescent or glabrous, green or purple. Main stem rises from first and high order buds, during the growth of mother plant. Branches rise from below ground nodes on the main stem. Slender leafy branches develop frequently from stems. Above ground maxillary branches may have anthocyanin pigment and spread erect giving a close appearance or may spread out giving an open appearance.

The tuber is a thickened stem having the cells mostly filled with starch as a reserve food for the new plants. The eyes are the promise of the future branches. The skin differs from the surface covering of the rest of the plant by being formed of a layer of delicate cork with its accompanying

lenticels (respiratory structure) like stem of other plants. Fibrous framework as well as pith, is continued from the leaf. Tubers are liquid more from part to part most rapidly through the tissue and not through the proper faith.

The development of and growth of potato can be divided into 7 major phases e.g., seed germination and emergence, tuber dormancy, tuber sprouting, emergence and shoot expansion, flowering, tuber development, and senescence (Jefferies and Lawson, 1991). The seed germination and emergence are the starting point for plants derived from true potato seeds (TPS). During this stage the seed absorbs water and becomes swollen, afterwards, radicle and plumule emerge. Gradually shoot emerges above the ground. On the other hand, plant grown from tubers are must go through tuber dormancy which is a physiological state of the potato tubers when autonomous sprout growth will not occur from the tubers even after providing favorable conditions for sprouting (Reust et al., 2001). After the dormancy period, sprouting will happen if ideal germination conditions are provided (darkness with a temperature of 15-20 ºC and 90% relative humidity). After successful sprouting shoots emerge from soil and expands. The next stage, flowering, is not an essential stage in potato growth. However, flowering followed successful pollination leads towards berry formation where TPS can be obtained. The tuber development is an important phase of potato growth where the stolon swells and forms tuber. At last, the senescence occurs starting from yellowing of leaves to death of the stems, ultimately falling on the ground.

1.1.1 Origin of potato

Potatoes are thought to have been produced independently several times and were largely produced in the United States by the Incas 1,800 years ago. Under the Spanish invasion, potatoes were introduced to Europe during the second half of the 16th century. By the end of the 17th century, the plant was a major crop in Ireland, and by the end of the 18th century, it was a major crop in continental Europe, especially in Germany, and in western England. It continued to spread, in the twentieth West and East, during the first forty years of the 19th century, and the Irish economy itself relied on sweet potatoes. However, the catastrophic failure of Irish crops in the middle of the 19th century (especially in 1846 and 1848), due to previous severe damage (*Phytophthora infestans*), and the Irish Potato Famine produced a very cautious crop dependence situation (Encyclopedia Britannica).

The potato diffused widely after 1600, becoming a major food resource in Europe and East Asia. Following its introduction into China toward the end of the Ming dynasty, the potato immediately became a delicacy of the imperial family. After the middle period of the Qianlong era (1735–96) in the Qing dynasty, population increases and a subsequent need to increase grain yields coupled with greater peasant geographic mobility led to the rapid spread of potato cultivation throughout China, and it was acclimated to local natural conditions (Boomgaard, 2003).

In India, Edward Terry mentioned about potato in his travel accounts of the banquet at Ajmer by Asaph Khan to Sir Thomas Roe, the British Ambassador in 1675. The vegetables gardens of Surat and Karnataka had potatoes as mentioned in Fyer's travel record of 1675. The Portuguese introduced potatoes, which they called 'Batata', to India in the early seventeenth century when they cultivated it along the western coast. British traders introduced potatoes to Bengal as a root crop,

'Alu'. By the end of the 18th century, it was cultivated across northern hill areas of India (Nunn and Qian, 2011). Potatoes were introduced to Tibet by the 19th century through the trade route from India (Gopal and Srivastav, 2008).

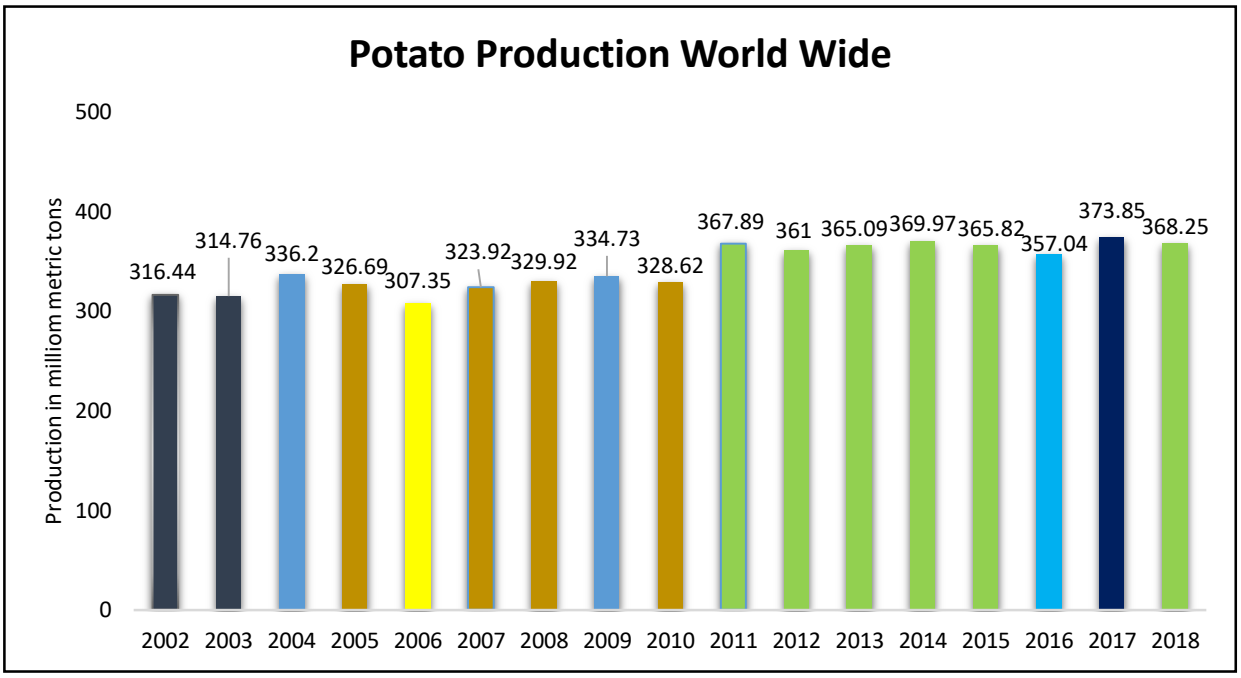
1.1.2 Nutritional status of potato

Potato is an excellent source of carbohydrate with low-fat and high fiber content. It contains 75% water, 21% carbohydrate (of which 82% is starch), 2.5% protein and below 1% fiber (Bradeen and Haynes, 2011). It has higher protein content and dry matter in comparison to cereals grown in similar unit of area (Bamberg and del Rio, 2005). A medium sized boiled potato contains high amount of Ca and also other minerals (Fe, Zn, K) in significant quantity. It also has several trace elements in substantial amount, e.g., Mn, Cr, Mo, and Se. However, the vitamin and mineral content of potato greatly varies with the flesh color of potato. The flesh of potato can be white, purple, yellow red and blue flesh (CIP, 2011). The yellow flesh is results from the presence of carotenoids whereas presence different levels of anthocyanin's results in red, purple and blue flesh. These pigments are antiaging agents.

1.1.3 World-wide potato production

Potato is consumed daily by billions of people around the world. It is a critical crop in terms of food security for exponentially growing population and it is forecasted that 50% of the extra food required to feed the population of the world will be covered by potato alone (Birch et al., 2012). Half of the total produced potato worldwide is consumed fresh while rest of the portion is processed into food products and ingredients, animal feed, or used as seeds to sow in the following season.

Previously potato was considered as a crop of developed countries but recently the production significantly increased in the developing nations. According to the Food and Agricultural Organization the potato production increased by 12.5 percent from 2008 to 2018 from 327.19



Graph 1.1: statistic of worldwide potato production from 2002 to 2018 in million metric tons

million tons to 368.17 million tons (FAOSTAT, 2020). Previously, Europe was the largest producer of potato but the production has dropped in this continent from 121.61 million tons in 2008 to 105.18 million tons in 2018, whereas, in Asia, which is the currently the largest potato growing region, showed an increased potato production from 142.30 million tons in 2008 to 188.64 million tons in 2018 (FAOSTAT, 2020).

China is the largest producer of potato in the whole world with a gross production of about 90.3 million tons followed by India with a total production of 48.5 million tons (FAOSTAT, 2020). Other potato growing countries ranked in top ten are Ukraine, Russian Federation, United States of America, Bangladesh, Germany, France, Poland and Netherlands. However, in terms of export, Netherlands is the largest exporter of potato. In 2018, this country exported potato worth about

800 thousand USD (FAOSTAT, 2020). Other notable potato exporting countries are France and Germany. In Asia, China is the largest exporter with an export value of approx. 261 USD. In contrast, Belgium is the largest potato importing country with an import value of around 540 thousand USD.

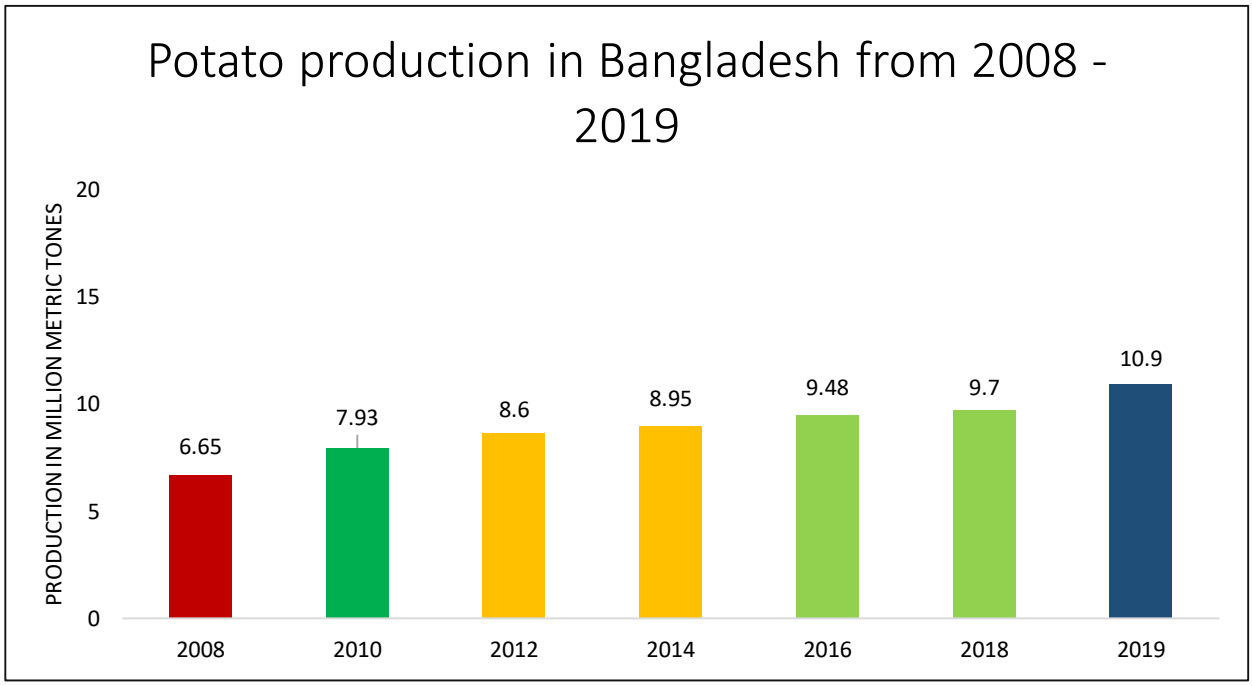
1.1.4 Potato production and cultivation in Bangladesh

Potato is the third most important crop in Bangladesh after rice and wheat. It is the most grown vegetable crop of Bangladesh. Sandy loam and loam soil are considered best for potato cultivation in Bangladesh. High to medium-high lands with proper sunlight, irrigation and drainage system should be chosen for best yield during potato cultivation. In Bangladesh, November is the best time to sow potatoes.

Tuber Crop Research Centre (TCRC) under the Bangladesh Agricultural Research Institute (BARI) is the main governing body of potato variety development and germplasm maintenance. Till date, 94 high yielding varieties (HYVs) of potato have been released by TCRC (Mia, 2016) with various aims and objectives. Most of them are exotic and most of them are collected from Netherlands and International Potato Center (CIP). Few are collected from Germany, India, Scotland and France. Remaining are developed by TCRC through hybridization of these collected materials (Kawochar et al., 2014). Among all, BARI Alu-7 (Diamant), BARI Alu-8 (Cardinal), BARI Alu-13 (Granola), BARI Alu-25 (Asterix), BARI Alu-28 (Lady Rosetta) and BARI Alu 29 (Courage) are the most popular ones.

In spite of low yield, the indigenous potato varieties are popular among the growers and consumers primarily because of their good keeping quality under ordinary room conditions, low cost of production, ability to give reasonably lower yield under lower input conditions, high market

demand and taste. Moreover, the indigenous potato varieties fit well into the production system of small and marginal farmers. The indigenous potato varieties are grown mostly in



Graph 1.2: Statistic of potato production in Bangladesh from 2008 to 2019 in million metric tons (Source DAE)

north-west and north-eastern districts of Bangladesh. Most of the indigenous varieties are confined in Rangpur, Bogra, Joypurhat, Mymensing, Sherpur, Netrokona, Naogoan, and Gaibanda district. The number of varieties grown in this country and their distribution in different areas are not precisely known. Some popular Indigenous potato verities of Bangladesh are Ausha, Challisha, Dohazari Sada, Festa Shill, Hagrai, Lal Pakri, Lal Shil, Patnai, Sadaguti, Shill Bilati, Surjamukhi (Kawochar et al., 2014).

The potato production of Bangladesh has increased remarkably from 6.65 million tons in 2008 to 9.47 million tons in 2016 (FAOSTAT, 2020). According to the Bangladesh Bureau of Statistics (BBS), in the fiscal year 2018-19, Bangladesh produced 9.66 million tons of potato from 1158 thousand acre of land with a yield potential of 3376 kg/acre (BBS, 2019). Now-a-days, Bangladesh

has a surplus in potato production than the local demand. However, still potato is considered as vegetable only in Bangladesh. Recently, industries are being developed as the demand of chips, crisps, flakes and French fries are increasing in the country day by day. Bangladesh is ranked 6th in the world for total potato production however, it only exported about 25 thousand tons of potatoes in 2018 with an export value of only 5744 thousand USD (FAOSTAT, 2020). Such poor performance in export instead of having high gross production of potato is resultant of quality deterioration, particularly, due to disease infestations.

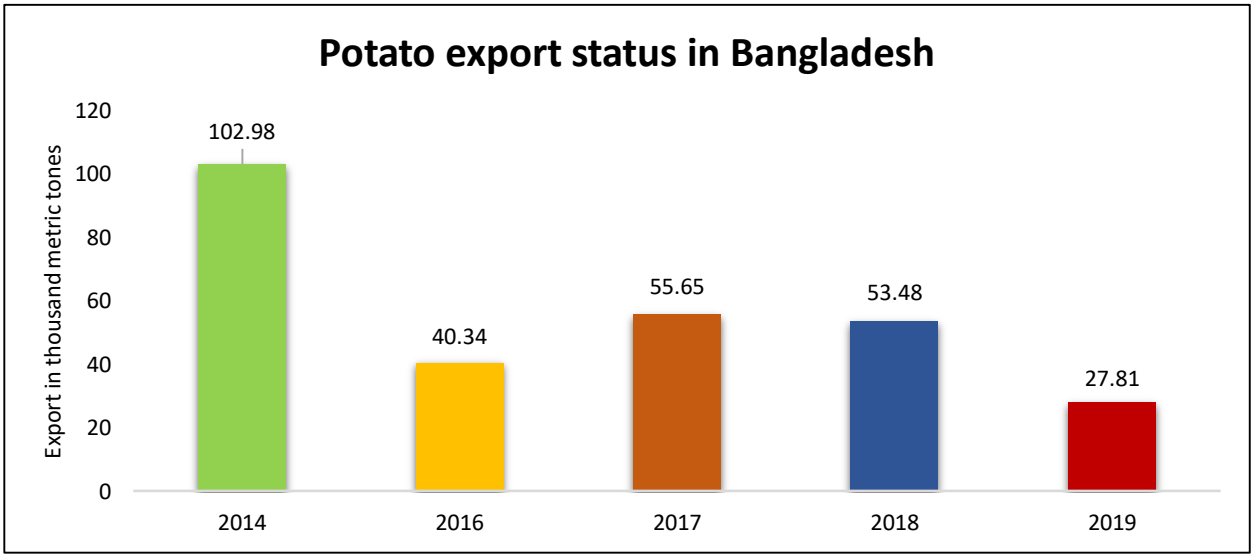
1.1.5 Problems with potato production in Bangladesh

The major problems of farmers for potato cultivation in Bangladesh are Non-availability of quality seeds timely, High price of fertilizer, Lack of irrigation facilities and high cost, Lack of credit facilities, Insect pest and disease problem, Labour crisis, Lack of storage facilities, High cold storage charge, Marketing problem, No purchase by the government, Lack of cash capital (Uddin *et al*, 2010, Rashid et al., 2001, and BARI Annual Report, 2009).

In 2008, about 8 million metric tons of potatoes have been produced from 0.5 million hectares of land in Bangladesh. It is reported in different newspapers that 5 thousands of tons of potatoes are going to rot due to lack of adequate cold storage facility. If this loss can be minimized, it will reduce the food shortage of Bangladesh to a great extent. Hence, potato may prove to be a useful tool to achieve the nutritional security of the nation (Ayub and Monayem, 2009).

1.1.6 Potato production and export from Bangladesh

Since 1974, potato exports have been less than 150 tons in every year. Table potatoes have been shipped primarily to Penang (Malaysia) and Singapore, but also to the Gulf States. Given the rapid increase in potato production and the limited exports to date, various observers have noted the interest on the part of government officials, representatives of the cold storage industry and independent traders in expanding exports as a means of expanding the market for Bangladesh



Graph 1.3: Statistic of export of potato in Bangladesh from 2014 to 2019 in thousand metric tons (Source DAE)

potatoes, increasing receipts of hard currency, and broadening the economy's export base (Elias *et al*., 1984). Table potatoes grown from improved seed are most suitable for export and are mostly produced either on a contract farming basis or the exporter leases land to grow them. Some larger exporters are however buying potatoes from the open market. Processing varieties are also usually grown through contract farming or on land leased by the processor. Since 2001, the government has intervened in export activities by paying a 20 per cent cash incentive for potato exporters. In 2010 this incentive was made applicable all year round; before then it was only for a period of

three months (February-April). The government has recently been confronted with demands from exporters to double the incentive. They argue that other exporting countries in the region (such as India, and in particular Pakistan) are subsidizing their exporters up to levels of 40 to 60 per cent.

1.2 Importance and advantages of potato production

Potato is the third largest food crop grown in Bangladesh and mainly consumed as vegetable. Various food items such as singara, samucha, chop, chips etc are also made from potato. Adequate supply of potato stabilizes the vegetable market round the year (Moazzem and Fujita, 2004).

Bangladesh produces substantial quantity of potato every year. Total production of potato was 6648 thousand tons from the area of 400 thousands hectares in 2007-08 (BBS, 2009). Though Bangladesh has become a major potato producer in the SAARC countries, the status of this crop has remained vegetable in the country. One of the major problems faced by developing countries in general and Bangladesh in particular, is the ever increasing population. As per the current trend, the population in Bangladesh is expected to be around 172.90 million by the year 2020. For further increase in agricultural production, the only option is to grow high productivity crops, like potato. We have been relying heavily on the major cereal crops like rice, wheat and maize to feed the ever increasing population of the country. Such an over dependence on cereals should be reduced gradually if we have to ensure food security, in the decades to come. Potato can help to widen the food supply base and thereby help to minimize the risk of serious food shortages in the tropics and sub-tropics.

A developing country like Bangladesh needs not only the quantity of food but sufficient quantity and quality of a balanced nutritious food. It is a fact that if the food available provides balanced

nutrition, the quantity of food intake is relatively low, e.g. in developed countries, where people consume balanced food and their dietary intake is relatively low in quantity. Whereas, in the developing countries, the food availability is not well balanced, consequently the quantity of dietary intake is higher because people tend to eat much cereal (mainly rice) to compensate for the poor nutrition. This results in greater demand for food and higher pressure on the limited land available to produce required quantity of food (Azimuddin *et al*, 2009).

Potato cultivation offers many benefits to farmers and the wider population. Potatoes are healthier than rice as they also contain protein, fiber and vitamin C in addition to starch. In tropical areas, potatoes are currently mainly eaten by growers and their families, with only a small part traded and able to reach the population in cities. This is unfortunate as there is considerable demand for potato. A growing and increasingly affluent middle class likes to eat crisps and French fries. Hotels, too, need to provide for this demand. As a consequence, potatoes are imported at high cost. Growers could therefore get high prices for potatoes that are especially suitable for processing into crisps and fries.

The potato is a profitable crop in tropical countries. It has higher yields and is more nutritious than white rice. Despite this, production levels remain very low. Farmers, governments and plant breeding companies are insufficiently informed about the complex cultivation process.

Short duration and wide flexibility in planting and harvesting time are potatoes other valuable traits that help adjusting this crop in various intensive cropping systems without putting much pressure on scare land and water resources. Potato is grown during winter season for which it is less susceptible to the monsoon rain and floods. The advantages of the potato over other crops are: It's potential for high productivity, its potential for being extremely profitable and easily marketed; its price is relatively stable.

1.3 Potatoes planted from seed have the following growth stages

Few crops are as rewarding to grow as potatoes. From watching their little eyes open and emerge from the soil after planting to peaking around the base of the plants to see the first tubers forming to finally harvesting a bountiful crop of fresh potatoes. The various stages of growth of potato plantlets are explained.

1.3.1 Sprout development stage

This stage begins with several eyes sprouting when the tuber is in storage, continues through planting and up until shoots emerges from the surface of the soil. The time involved for the shoots to emerge from the ground varies greatly depending on the length of the shoot, moisture in the soil and other environmental conditions. With a sprout at the ideal length of 1-2 cm, shoots should begin emerging from the ground at around 21-30 days after planting (DAP). During this stage the plant still uses nutrient reserves stored in the tuber.

1.3.2 Vegetative growth stage

This stage shows rapid growth of leaves, stems, new shoots and roots. The plant still relies on food reserves stored in the seed tuber, but has already begun to take small quantities of nutrients from the soil. Generally it occurs between 30- 50 DAP.

1.3.3Tuber initiation stage

Although tuber-forming roots begin to form during the vegetative stage, formation of actual tubers only occurs at 40-55 DAP. This stage takes place over a relatively short period of about 10-15 days. Tubers formed after 65 DAP will not reach optimum size when harvested. Plants require nutrients in large quantities during this stage.

1.3.4 Tuber bulking stage

At this stage the plant itself has already stopped growing, and only the tubers become any larger. Normally this stage occurs at 50-80 DAP.

1.3.5 Tuber maturation stage

Yellowing leaves and drooping stems characterize this stage. Tuber skins gradually harden and do not pare away easily. Tubers harden due to their increased starch content. The best time to harvest a plant is at over 100 days old, because tubers will have reached maximum maturity signified by their hardness and sturdy skins.

1.4 Modern potato varieties

In the previous couple of decades, several dozens of high yielding varieties (HYV) of potato were delivered to Bangladesh and tried experimentally under local conditions before they were recommended for general cultivation. During the 1970s, close to 16 varieties were initially selected but subsequently 10 were dropped from those. Through constant evaluation of the traits, varietal performance, and considerations of other characteristics, about 10 HYV are released for cultivation within the country. However, huge amount of potato seeds are imported per annum by the Bangladesh Agricultural Development Corporation (BADC) for distribution among farmers. For the production of HYV seed potatoes Bangladesh Agricultural Research Institute (BARI) has also established a farm at Debiganj in Panchagar district. Among the high yielding popular varieties, some notable varieties are: (a) Cardinal- probably most popular among the foreign varieties with oblong and reddish tubers, shallow eyes, and smooth skin. The variety has been introduced from

Holland and has yield potential of 20-25 m tons per ha; (b) Diamant - another Holland- variety with oval to oblong shape, pale yellow tubers, smooth skin, and shallow eyes. It is quite disease resistant. Per hectare yield ranges from 18-24 m tons; (c) Kufri Shindhury - tubers are reddish and round, and eyes are deep with rough skin. This variety was introduced from India and is comparatively less susceptible to pests and diseases. It has a yield potential of 18 to 22 m tons per ha. Other notable exotic varieties are Patronis, Alpha, Archa, Multa, Ukama, Hira, Maurin, Origo, Alisa, etc. In recent years, the Tuber Crops Research Centre of BARI has collected many new sorts of potato from the International Potato Research Centre, Peru and from other sources. These are being tested under Bangladesh field conditions to determine whether they can be recommended for commercial cultivation in the country. The Centre has already made good contribution towards the event of some high yielding potato varieties. (Khalil et al., 2014)

1.5.1 Indigenous Potato variety

Varieties which were introduced long before from the different parts of the world and became degenerated and have lost their identity, but are still under cultivation under different popular names known as indigenous varieties (Siddique, 1995).

In the Andes of Bolivia, Ecuador and Peru, farmers grow varieties of potatoes that are never seen outside this region. These native Andean potatoes display extraordinary diversity of taste and texture and come in a fascinating array of shapes and colors; they are quite different from the white-fleshed, smooth-skinned and relatively bland varieties commonly grown in the rest of the world. For perhaps as long as 8000 years these native potatoes have been important in the nutrition

and economy of Amerindian subsistence farmers, but they have been neglected by Science and largely ignored by the potato and health food industries.

1.5.2 Indigenous potato varieties of Bangladesh

The potato varieties introduced in Bangladesh many years ago and adapted to the local conditions are known as indigenous potato varieties (IPVs) which cover approximately 40% of the area under potato cultivation in the country. The yield potential of IPVs is low but its demand as well as price of IPVs is high in the market comparative to other modern varieties (Siddique and Hussain, 1988). The indigenous potato varieties are also known as local potato or Deshi Alu varieties in Bangladesh.

The total varieties of IPV grown in our country are unknown and nomenclature is also sometimes confusing. Interestingly one particular variety of IPVs is known as different name in different areas. Again different varieties of IPV are grown in different places depending on the local popularity such as Dohazari Lal has popularity in Chittagong and it is grown in some pericular areas of Chittagong.

The common IPVs in Bangladesh are given below (Siddique, 1995),

- Ausha
- Challisha
- Dohazari Lal
- Fretashil
- Hagrai

- Lal Pakri-01
- Lal Pakri-02
- Lal Shil
- Patnai
- Shadaguti
- Shilbilati
- Sujamukhi

1.5.3 Yield of IPVs in Bangladesh

The national average yield of IPVs is about 7.2 tons/ha whereas the modern potato varieties average yield is about 11.7 tons/ha. The lower yield of IPV is mainly due to the cultivation of some varieties having lower yield potential and use of poor quality seed and sub-optional production practices. The IPVs frequently show the symptoms of different virus infection. Virus infected "Yellows" are the common occurrence. Plants showing such symptoms give extremely poor yields (Siddique, 1995).

1.5.4 Scope of improving IPVs using Biotechnology

Yielding ability of the local varieties of potato can be improved through a process of positive selection and through rouging of plants associated with "Yellows. Again yield of the indigenous potato varieties can be increased by the different management practice and a package of practice by which the yields of these varieties can be significantly enhanced is now available. The package includes use of better quality seed tubers collected from apparently healthy plants, planting of

medium and large sized seed tubers, early planting, judicious manuring and optimal irrigation or cover mulching.

The main causes of yield reduction in Bangladesh are lack of quality seed potato, pathogen infected seed potato used, low yielding variety used and faulty management. It is proved that use of quality seed potato may produce 20-30% high yield. However, at present there is no alternative way of high yielding variety developed in the world. Because agricultural land has decrease and the people increase.

At this situation Biotechnology can be played a vital role to improve the IPVs. By using biotechnological tools, already improvements of IPVs have started. Way of improving IPVs such as,

- Plant tissue culture technique for disease free seed production.
- *In vitro* meristem culture for the development of virus free plant materials.
- Induction of micro-tuber for rapid production of virus free plant materials.
- Induction and evaluation of somaclonal variation were employed for the varietal improvement.
- Doubled haploid plant production through *in vitro* techniques.

1.6 Major diseases of potato in Bangladesh:

Solanum tuberosum is known to interact with a wide variety of fungi, bacteria, nematode, and insect species, many of which are important pests and disease-causing agents. Several publications provide extensive reviews of fungal and fungal-like (Forbes and Landeo, 2006; Termorshuizen, 2007), bacterial (Gopal and Khurana, 2008), virus and viroid (Jeffries et al., 2006; Valkonen, 2007), insect and nematode (Abdelhaq, 2006; Mugniéry and Phillips, 2007; Radcliffe and Langnaoui, 2007) pests of *S. Tuberosum.* A list of major potato diseases and their symptoms are presented in Table 1.

SL.	CATEGORY	NAME OF DISEASE	SYMPTOMS
1.	Bacterial	Bacterial ring rot	• Wilting stems and leaves • Dying leaves • Lower leaves wilting first • Ring of creamy yellow to brown rot visible when tuber is cut crossways
		Blackleg (Soft rot)	• Small, water-soaked lesions on base of stems originating from seed piece • Lesions may enlarge to form a large extended lesion stretching from base of stem to canopy • Tissue becomes soft and water-soaked and can be light brown to inky black in color

		Common Scab	• Raised brown lesions on tubers with corky texture • Deep, pitted brown or black lesions on tuber with straw-colored translucent tissue underneath
2.	Fungal	Black Dot	• Small black dots (fungal fruiting bodies) on tubers, stolons and stems • Roots may rot below ground • Leaves may turn yellow and wilt; infection may cause defoliation
		Black Scurf & Rhizoctonia Canker	• Flat, irregularly shaped black or dark brown fungal fruiting bodies on tuber surface • Tubers may be mishapen; red-brown to black sunken lesions on sprouts • Lesions may girdle the main stem causing leaves to curl and turn yellow

		Gray Mold	• Flowers covered in gray, fuzzy mold • Wedge shaped tan lesions on leaves • A slimy brown rot may be present on stems, originating from the petiole • Infected tubers have wrinkly skin and tissue underneath is soft and wet • Tubers often develop a gray fuzzy growth
		Pink Rot	• Stunted plant growth; wilting leaves • Dying leaves; marked tuber decay • Dark brown eyes on tuber • Cut tuber turns pink after 20-30 min air exposure, then turns brown and finally black
		Potato Early Blight	• Dark lesions with yellow border which may form concentric rings of raised and sunken tissue on the leaves and stems • Lesions initially circular but become angular • Leaves become necrotic but remain attached to plant

		Verticillium wilt	• Early death of plants • Leaflets dying on only one side of the petiole or branching stem • Cut through the stem reveals a discoloration of the tissue • Discoloration of tubers at stem-end
3.	Oomycete	Potato Late Blight	• Irregularly shaped spreading brown lesions on leaves with distinctive white fluffy sporulation at lesion margins on the underside of the leaf in wet conditions. In dry condition the lesions dry up and go dark brown with collapsed tissue • Water-soaked dark green to brown lesions on stems also with characteristic white sporulation; later in infection leaves and petioles completely rotted • Severely affected plants may have an slightly sweet distinctive odor • Red-brown firm lesions on tubers extending several centimeters into tissue • Lesions may be slightly sunken in appearance and often lead to secondary bacterial rots.

4.	Virus	Potato Leaf Roll Virus (PLRV)	• Young leaves rolled and yellow or pink • Lower leaves have leathery texture and roll upward; necrotic netting in vascular tissue of tuber may be present • Plant exhibits an upright growth habit and growth may be stunted
		Potato Virus A (PVA)	• Mild mosaic pattern or mottling on leaves • Severely infected plants may have alternating patches of yellow and dark green tissue • Leaves may have a shiny appearance • Stems bending outwards slightly
		Potato Virus X (PVX)	• Mild mosaic pattern on leaves • Severely infected plants may be dwarfed with smaller leaves • Necrosis of plant tops and tubers may occur
		Potato Virus Y (PVY)	• Symptoms vary widely from mild mosaic of leaves to leaf necrosis and plant death depending on the variety of potato and the strain of the virus • Leaves may turn yellow and drop from plant • Symptoms may be present on only one shoot of the plant

5.	Pests	Aphids	• Small soft bodied insects on underside of leaves and/or stems of plant • Usually green or yellow in color, but may be pink, brown, red or black depending on species and host plant • If aphid infestation is heavy it may cause leaves to yellow and/or distorted, necrotic spots on leaves and/or stunted shoots • Aphids secrete a sticky, sugary substance called honeydew which encourages the growth of sooty mold on the plants

Table 1. List of major potato diseases and their symptoms

Among all the diseases of potato the late blight disease, caused by *Phytophthora infestans,* is the most devastating one. Late blight of potatoes was responsible for the Irish potato famine in the mid-nineteenth century that caused death of millions of people. P. infestans was probably introduced to the United States from central Mexico, which is its center of origin. After appearing in North America and Europe during the 1840s, the disease spread throughout most of the rest of the world during subsequent decades and had a worldwide distribution by the beginning of the twentieth century. Severe late blight epidemics occur when P. infestans grows and reproduces rapidly on the host crop. Reproduction occurs via sporangia that are produced from infected plant tissues and is most rapid during conditions of high moisture and moderate temperatures (60°-80°F). Sporangia disperse to healthy tissues via rain splash or on wind currents. Reproduction is

asexual; each sporangium is an exact copy of the one that initiated the parent lesion, and each can initiate a new lesion. During the early 1990s several exotic strains of *P. infestans* were introduced from Mexico. These strains have increased the severity of late blight on potato and tomato because they are more aggressive than earlier ones in the United States and Canada. They initiate infections more quickly and reproduce more profusely, causing epidemics to occur rapidly.

1.6.1 Late Blight potato disease:

Late blight of potatoes was responsible for the Irish potato famine in the mid-nineteenth century. Before the disease appeared in Ireland it caused a devastating epidemic in the early 1840s in the northeastern United States.

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Fig.2. Effect of LB in a potato field

are produced from infected plant tissues and is most rapid during conditions of high moisture and moderate temperatures (60°-80°F). Sporangia disperse to healthy tissues via rain splash or on wind currents. Reproduction is asexual; each sporangium is an exact copy of the one that initiated the parent lesion, and each can initiate a new lesion.

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In Bangladesh, late blight causes serious yield loss in potato every year. Late blight was first reported in Bangladesh in 1922. The disease damages minimum 25 per cent of the potato production a year, according to Bangladesh Agricultural Research Institute (BARI) (Wardad Y. 2018). In recent years more than 300 varieties, 2050 germplasm lines and 250 true potato seed (TPS) progenies were evaluated for resistance to major diseases under field conditions. Screening for late blight resistance was carried out under both natural and artificial conditions. So far more than 300 germplasm (exotic and local) and TPS progenies were screened through artificial inoculation (Hossain M. 2009).

1.6.2 Control measurement:

Use of integrated management practices is important for successful suppression of potato or tomato blight. In the absence of sexual reproduction P. infestans requires a living host to survive between seasons. Therefore, sanitation (elimination or exclusion of infected plant parts from a farm) is vital within the overall management strategy. Ideally, no infected potatoes should be

present within the vicinity of the crop. Volunteer plants which may be infected should be destroyed. Cull potatoes should be frozen, crushed, fed to livestock, or buried under a minimum of 2 feet of soil. Only tubers that are free of P. infestans should be planted. The "Certified" grade for seed potatoes allows up to 1 percent late blight infection. Growers should request information from the seed potato producer on whether blight was observed during field or harvest inspections.

Regular inspections of growing crops are important to the overall management of late blight. Because topography and crop growth can influence the microclimate encountered by the pathogen, late blight may be detectable earlier in some areas than in others. It is likely to appear first in wet areas (low spots in the field, areas adjacent to woods and hedgerows, dense crops, or areas adjacent to other features that might shade crops), especially when the macroclimate has been but optimal for pathogen development.

Protectant fungicides are often needed from mid- to late season when plants are growing actively and have a dense canopy. Applications should be repeated regularly to replace fungicide that has been washed or abraded away and to protect foliage produced since the last application. It is during this time that the more effective fungicides are needed. Fungicides that have systemic activity (penetrate into plant tissues) are necessary if a crop has been exposed to sporangia within the last 24 hours. Even if the first infections occurred more than 24 hours earlier, if lesions are visible in the crop and a systemic has not been applied, an effective systemic will probably provide some benefit that is not possible from a protectant. The summary is successful management of late blight relies on an integration of the following tactics: removing sources of the pathogen by eliminating cull potatoes and volunteers and planting only healthy seed tubers; using resistant cultivars when possible and as they become available; scouting locations where late blight might appear first; using a forecasting scheme to gain early warning of weather that is favorable to disease and to

adjust frequency of fungicide application or the intensity of scouting; and using appropriate protectant or systemic fungicides. After harvest, potato tubers are to be stored at cool temperatures under conditions sufficiently dry that there is no free moisture on tuber surfaces. Control tactics are constantly modified as new information and technology become available.

1.7 Potato Breeding:

The principal advantage of breeding clonally propagated crops is that each clonal variety is fixed and simple to maintain. Genetic purity is less of an issue in vegetatively—than in sexually—propagated crops. One substantial disadvantage of vegetative propagation though is that diseases are easily transmitted across clonal generations during propagation; another is that potato planting material is bulky and perishable by nature and the production of healthy material is expensive.

In the course of potato breeding an "F1 clone hybrid" is generally crossed with another "F1 clone hybrid," and the progeny is heterogeneous and has an extreme large segregation variance. A good parent generates a large genetic variation around a high family mean for a given trait. Once heterogeneous and heterozygous progenies are generated by crossing two potato clones, selection of clones within a given pool of genetic variation for variety development is conceptually, if not technically, straightforward. All the genetic advantages of clonally propagated crops can be used for variety development, and the genotype that will finally be released is among the progeny immediately after the initial crossing (Bonierble M. et. el. 1970)

1.7.1 Potato Genetics and Breeding Problem

Generally the term potato refers to all the tuber-bearing *Solanwn* species in addition to the commonly known *Solanuin tuberosum* L. Genetically, the potato is a complex and diverse group of tuber-bearing species and subspecies belonging to the genus *Solarium.* Collectively, over 160 species are known (Hawkes, 1978) but members of *Solarium tuberosum L.* (mainly the subspecies tuberosuin) occupy most cultivated acreage worldwide. Virtually all commercial cultivars are extremely heterozygous tetraploids (2n=4X=48) (Ugent, 1970), vegetatively propagated and as such pose several problems for plant breeders. These include the high level of heterozygosity, the common occurrence of pollen sterility, incompatibility, selection difficulties in the seedling and first clonal years, build-up of viruses, and difficulties in germplasm storage (Miller and Lipschutz, 1984).

1.7.2 Potato breeding for late blight resistance:

Potato blight is a significant global constraint to potato production. Potato late blight has been the most important pathogen of the potato crop. The disease is characterized by huge destruction and decay of the tubers. Potato blight affects the health of foliage and tubers limiting profitable potato production. Significant financial costs in terms of crop protection and crop losses are incurred when intervention measures to control potato late blight are unsuccessful. One aspect of late blight disease management in the field is the use of resistant cultivars. It is important to draw upon many germplasm resources to develop a broad genetic base and identify differences in expression of single components of field resistance (i.e. resistance to infection, spread and sporulation), then hybridize consistent with complementary components.

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1.8 LB Resistant Genes of potato:

Phytophthora infestans is the causative agent that can destroy all potato parts, including leaves, stems and tubers (Fry, 2008), and is the main threat to potato production and responsible for 16% of yield losses globally (Haverkort et al., 2016). Similar to other crops, disease management using resistant varieties is one of the most effective strategies, environmentally and economically, to control late blight disease (Fry, 2016). Plant immunity is activated by detecting conserved microbial molecules, microbe (pathogen)-associated molecular patterns (MAMPs or PAMPs), known as pattern-triggered immunity (PTI), as well as by detecting the pathogen effectors, known

as effector-triggered immunity (ETI) (Jones and Dangl, 2006). Plant pathogens can successfully colonize plant hosts by delivering effector proteins that repress host immunity and increase disease severity (Turnbull et al., 2017). In turn, few effectors might be recognized by the correspondent resistance (R) proteins, triggering a rapid immune response known as effector-triggered immunity (ETI), which often leads to an hypersensitive response (HR) cell death (Jones and Dangl, 2006; Turnbull et al., 2017).

To achieve effective control of late blight, potato breeders have to adopt novel techniques and strategies in various aspects such as detection and identification of new Rpi genes, their introgression and field application (Vleeshouwers and Oliver, 2014). Great efforts were made at the beginning of the last century to introgress Rpi genes into potato varieties from the wild Mexican species Solanum demissum to provide resistance to P. infestans in the cultivated potato S. tuberosum. This also led to the development of differential potato lines with 11 distinct recognition specificities, called R1-R11 (Black et al., 1953; Malcolmson and Black, 1966). Many R genes (Rpi) have been identified, cloned and some of them were introgressed into potato cultivars from wild Mexican Solanum species (Goodwin et al., 1992; Grunwald and Flier, 2005; Goss et al., 2014), including R1–R11, R3a, R3b, R9a and Rpi-demf1 from S. demissum (Ballvora et al., 2002; Huang et al., 2005; Lokossou et al., 2009; Jo et al., 2015), Rpi-blb1(RB), Rpi-blb2, Rpi-blb3, Rpi-abpt and Rpi-bt1 from S. bulbocastanum (Song et al., 2003; Van der Vossen et al., 2003; Park et al., 2005; Van der Vossen et al., 2005; Lokossou et al., 2009), Rpi-sto1,2, Rpi-pta1,2, and Rpi-plt1 from S. stoloniferum (Vleeshouwers et al., 2008; Wang et al., 2008; Champouret et al., 2009), Rpi-amr3 from S. americanum (Witek et al., 2016), Rpi-mch1 from S. michoacanum (Sliwka et al., 2012a), and Rpi1 from S. pinnatisectum (Kuhl et al., 2001; Lokossou et al., 2010). Additionally, further R genes have been identified in another center of genetic

diversity of tuber-bearing Solanum, the Andean region in South America, such as Rpi-vnt1 from S. venturii (Foster et al., 2009; Pel et al., 2009), Rpi-mcq1 from S. mochiquense (Smilde et al., 2005), Rpi-ber from S. berthaultii (Rauscher et al., 2006), Rpi-mcd1 from S. microdontum (Tan et al., 2008), Rpi-pcs from S. paucissectum (Villamon et al., 2005), Rpi-cap1 from S. capsicibaccatum (Jacobs et al., 2010), Rpi-rzc1 from S. ruiz-ceballosii (syn. S. sparsipilum) (Śliwka et al., 2012b; Brylińska et al., 2015) and Rpi-chc1 from S. chacoense (Zhu et al., 2015).

Much attention has been paid to RXLR effectors since the cloned avirulence protein (Avr) of oomycete pathogens belong to this type of effector, such as P. sojae Avr1b (Shan et al., 2004), Hyaloperonospora arabidopsidis ATR13 (Allen et al., 2004) and ATR1 (Rehmany et al., 2005), and P. infestans PiAvr3a (Armstrong et al., 2005). So far, over 10 Avr genes, identified by 10 cognate-recognition Rpi genes, have been described in P. infestans, including PiAvr1 (Van der Lee et al., 2001; Ballvora et al., 2002), PiAvr2 (Gilroy et al., 2011), PiAvr3a (Armstrong et al., 2005), PiAvr3b (Rietman et al., 2012), PiAvr4 (Van Poppel et al., 2008), PiAvr8 (Vossen et al., 2016), PiAvrblb1 (Song et al., 2003; Vleeshouwers et al., 2008), PiAvrblb2 (Van der Vossen et al., 2005; Oh et al., 2009), PiAvrvnt1 (Foster et al., 2009; Pel et al., 2009), PiAvrSmira1 and PiAvrSmira2 (Rietman et al., 2012; Yoshida et al., 2013) as shown in (Supplementary Table S1). P. infestans is predicted to encode 563 RXLR effector genes which were mainly found located in repeat-rich or gene-sparse regions of the genome, meaning that more rapid evolution compared to other genes located in gene-dense regions (Haas et al., 2009; Yin et al., 2017). It might explain its ability to escape host defense mechanisms (Lenman et al., 2016). It has been well-documented that RXLR effectors play important roles in potato–P. infestans interactions. In rare cases of recognition by the cognate R genes, they mediate late blight resistance by triggering HR; in most cases, they are not recognized and function as typical

virulence factors by interfering with host cell structure and function, resulting in enhancing plant susceptibility (Huang et al., 2019).

There are generally two strategies to improve late blight resistance. The first is the deployment of many different R genes to offer tentative durable resistance since changes of multiple effectors are predicted to increase the penalty. The second strategy is to identify genes that are capable of recognizing various effectors or core effectors. In fact, the identified R genes from varieties that showed durable disease resistance were confirmed to be able to recognize two or more effectors.

1.8.1 Late Blight Resistant Genes SSR Marker

Marker	**Chromosome**	**Target gene/QTL**	**Sequence (5'3') (F|R)** →		**ATm (°C)**	**Reference**
R2-F1/R3	4	Rpi-abpt	GCTCCTGATACGATCCATG	ACGGCTTCTTGAATGAA	50	Kim et al., 2012
R3b-F4/R5	11	R3b	GTCGATGAATGCTATGTTTCTCGAGA	ACCAGTTTCTTGCAATTCCAGATTG	55	Rietman, 2011
SHa	11	R3a	ATCGTTGTCATGCTATGAGATTGTT	CTTCAAGGTAGTGGGCAGTATGCTT	56	Huang et al., 2005
CosA	5	R1	CTCATTCAAAATCAGTTTTGATC	GAATGTTGAATCTTTTTGTGAAGG	55	Gebhardt et al., 2004
Q133	10	RPi-ber	ATCATCTCCTCAAAGAATCAAG	ATCTCCCCATTGACAACCAA	50	Tan et al., 2010
Ssto-448	8	Rpi-sto1	GTGGAACGCCGTCCATCCTTAG	TGCATAGGTGGTTAGATGTATGTTTGATTA	59	Sokolova et al., 2011
BLB1	8	Rpi-blb1	AACCTGTATGGCAGTGGCATG	GTCAGAAAAGGGCACTCGTG	58	Wang et al., 2008
BT1	8	Rpi - bt1	CTACATGGCTGTCATTCACT	CATAGGGCAACATTTAATCTC	53	Chen et al., 2017
BT1-F1/R1	8	Rpi - bt1	GAGATTAAATGTTGCCCTATG	GTTGGACAAAACTCAACTGAT	53	Chen et al., 2017
1521/518	8	Rpi - blb1	GAAAGTCTAGAGTTACACTGG	CAATCACAATGGCAGGAACC	58	Wang et al., 2008
45/XI	11	39956309..39958308	AGAGAGGTTGTTTCCGATAGACC	TCGTTGTAGTTGTCATTCCACAC	55	Tomczyńska et al., 2014

Table 2. List of late blight resistant gene SSR Markers

1.9 Objectives of the study

The main objective of this study is to produce disease-free high quality seed potatoes of selected indigenous potato varieties (IPVs), utilizing tissue culture technology. The specific objectives of the study are as follows:

(a) Production of TC-based plantlets of selected IPVs in a Tissue Culture Lab;

(b) Production of mini tubers (pre-basic seed potatoes) of selected IPVs under net-house conditions using the TC-derived plantlets;

(c) Production of potatoes of selected IPVs using the TC-derived mini-tubers.

Among the objectives (a) and (b) were described by Siddique *et al*, (2014). Objective (c) is under this study which is the main objectives of this study.

Chapter 2

2. Materials and Methods:

2.1 Setting of the experiment:

The research was carried out at Debiganj ASRBC Station (Panchagarh). One successful cross, ACI Pakri-1 (recipient) x LB Resistant donor variety, was obtained in 2015-16 season. Out of the plants raised from F^1 seeds in 2016-17 season, 72 individual plants were selected. The tubers obtained from the selected 72 plants were considered as the planting materials for planting in 72 lines during the 2017-18 season. In addition, tubers of 5 potato varieties, namely, ACI Pakri-1 (Recipient Parent), Donor Parent, Diamant (LB susceptible check), BARI Alu-40 (LB susceptible check) and BARI Alu-77 (LB resistant check), were considered as planting materials. Thus, there were 77 materials in total for planting and evaluation during the 2017-18 season.

Five tubers of each of 77 materials were planted for each level of LB inoculation treatment. A plant spacing of 60cm X 20cm was maintained. Normal cultural practices were applied. Well-sprouted tubers were planted on 14 November 2017. Each of the 77 planting materials were subjected to the following 3 levels of LB inoculation; (i) LB inoculation & No fungicide applied, (ii) No LB inoculation & No fungicide applied, (iii) No LB inoculation but Fungicide applied. Pencozeb and Acrobate MZ were used for the Fungicide application treatment. The fungicides were applied at 5 days interval, starting at 32 days after planting (DAP) and continuing up to 85 DAP. *Phytophthora infestans* inoculums was collected from ASRBC Laboratory, ACI Limited, Dhaka. The following steps were taken for LB inoculation: (i) Stage of application: 30 days after planting, (ii) Preparation: Each plant was covered with a transparent plastic bag before 24 hours of inoculation, and kept covered up to 48 hours after inoculation with *Phytophthora*, (iii) Inoculation: Inoculums was applied to cover all expanded leaves of test plants using a hand sprayer. Normal water was applied on check plants. Three plants from each line were inoculated,

(iv) Maintenance after inoculation: After establishment of infection, the plastic covers were removed, and the plants were misted 3 times a day at

10 a.m., 2 p.m. and 6 p.m. for 30 days, (v) Late blight severity was recorded as percentage (%) foliage area damaged on each plant at 30 days after inoculation. The stage of plant growth at which inoculums was applied, and plants covered with transparent plastic bag before 24 hours of inoculation are shown in Fig.3 (A) and 1(B), respectively.

Fig. 3. (A) Potato plants ready for LB inoculation at 30 DAP, (B) plants covered with transparent plastic bag before 24 hrs of inoculation.

2.2 Marker Assisted Selection (MAS):

In this present study Marker Assisted Selection (MAS) was conducted in selected 8 resistant lines along with parental materials. Five *P. infestans* resistance genes named Rpi-blb1 (Wang M et el., 2008), Rpi-bt1 (Oosumi T et el., 2009), Rpi-mcd1 (Tan MYA et el., 2010), Rpi-ber1 and Rpi-sto1 (Sokolova E et el., 2011) were used based on prior publications and total 5 primers were used for MAS. However, sprouts of tuber were used as explants for DNA isolation. Sprouting was initiated in freshly harvested tuber by 50mg/l GA_3 solution treatment. DNA was isolated by following CTAB method (Doyle JJ, 1990). PCR was performed with selected markers (Chen S et el., 2017).

Amplified PCR products were detected on a 3% agarose gel stained in 1 x Tris-base EDTA buffer and visualized on a UV trans-illuminator.

Gene name	Marker	Sequence	Annealing T^0C
Rpi-ber1	Q133	F:ATCATCTCCTCAAAGAATCAAG	56.5
		R:ATCTCCCCATTGACAACCAA	
Rpi-sto1	Ssto-448	F:GTGGAACGCCGTCCATCCTTAG	65.6
		R:TGCATAGGTGGTTAGATGTATGTTTG ATTA	
Rpi-abpt	R2-F1/R2-R3	F:GCTCCTGATACGATCCATG	52.5
		R:ACGGCTTCTTGAATGAA	
Rpi-blb1	1521/518	F: GAAAGTCTAGAGTTACACTGG	58.0
		R: CAATCACAATGGCAGGAACC	
Rpi-bt1	BT1F/BT1R	F: CTACATGGCTGTCATTCACT	56.0
		R: CATAGGGCAACATTTAATCTC	

Table 3. List of late blight resistant genes, respective markers, sequences and their annealing temperature for PCR amplification used in this study.

2.3. Recording of data:

The method for collection of data on LB infection and yield components & yield are presented below.

2.3.1. Data on LB infection:

Data were recorded at 10 days interval, starting from 1st inoculation or 1st visible lesion (Maria A et el., 2013).

1. Days to 1st lesion

2. Degree of sporulation (0-3 scale)

    0- Corresponds to no visible sporulation

    1- Represents very sparse sporulation

    2- Represents sporulation on few leaves

    3- Many sporangiophores being visible

3. Level of Infection

    a) 0 - No signs of infection.

    b) 0.1 - First single spore-bearing spots.

    c) 1.0 - Weak level of infection (5-10 lesions per a plant).

    d) 5.0 - About 50 lesions per plant; 1 of 10 leaf lobes is infected.

    e) 25 - Almost all leaves infected; plants are still in normal form. Field looks green.

    f) 50 - All plants infected; 50% of leaf area dead. Field still green with brown spots.

    g) 75 - Infection spread over 75% of leaf area. Field looks brown and green.

    h) 95 - Plants have only single leaves, but the stems are green.

    i) 100 - All leaves dead, and stems are dead or dry.

2.3.2. Data on tuber morphological characteristics:

Data on tuber color, tuber shape, eye color, eye deep/shallow and uniformity of the tuber were recorded.

2.3.3. Data on yield components and yield:

Data on days to 1st emergence, days to 80% emergence, number and weight of tubers per plant at harvest and yield of tubers per hectare were recorded.

2.4. Analysis of data:

Foliage destruction data was used for calculating Area Under Disease Progression Curve (AUDPC) followed by relative AUDPC (rAUDPC).

$$\text{AUDPC} = \sum_{i=0}^{n-1} [(X_{i+1} + X_i)/2] \, (T_{i+1} - T_i)$$

Where, "T" is the time of each reading, "X" is the percentage of affected foliage at each reading and "n" is the number of readings. The variable "T" can represent Julian days, days after planting or days after emergence (Yuen JE & Forbes GA, 2009)

Then, the relative area under the disease progress curve (rAUDPC) was calculated. This value was obtained dividing the AUDPC by the total number of days elapsed between the first and last evaluation of the foliage destruction (Jeger MJ & Viljanen-Rollinson LH., 2001)

rAUDPC= AUDPC/ (Difference between last evaluation and the first evaluation X 100)

Again, susceptible level was calculated from the rAUDPC value by calculating constant value and scoring highest value 9 for highly susceptible (Yuen JE & Forbes GA, 2009).

$S_X = S_y (D_x/D_y)$

Where, Sy and Dy represent, respectively, the assigned susceptibility scale value and observed disease measure (AUDPC or rAUDPC) for the standard genotype. Sx and Dx represent, respectively, the calculated susceptibility scale value and observed disease measurement for the genotype in question.

Finally, yield was calculated by the equation recommended by Tuber Crop Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI).

Chapter 3

3. Results and discussion:

3.1 LB infection:

There was no LB infection in the treatment where fungicides were applied at regular interval. Data on LB infection were collected from the treatments - LB inoculation & No fungicide applied and No LB inoculation & No fungicide applied. In general, similar results were obtained from both treatments. The 1st lesion was observed in all lines at 29 to 40 DAP and 80% or more infected plants were recorded at 32 to 46 DAP which was mostly similar to susceptible check, resistant check and parents.

On the basis of field evaluation, potato lines were selected under 3 categories, as follows, taking under consideration the level of LB infection and appearance of tuber : (a) LB resistant lines, (b) LB tolerant lines, and (c) LB susceptible, but with attractive appearance of tuber and high yield. Category-1 (LB resistant lines) includes lines showing high tolerance to LB under both levels of inoculums pressure.

The foliage destruction in the category was considered to be in the range of 1 to 25% at 85 DAP. In some lines, foliage destruction at 85 DAP was as low as in the LB resistant check variety BARI Alu-77. On the other hand, the recipient ACI Pakri-1 had 100% foliage destruction at 63-65 DAP. The donor had 58-65% foliage destruction at 85 DAP. The foliage destruction dramatically increased from 10% to 90% at 40 to 50 DAP in LB susceptible check varieties, Diamant and BARI Alu-40 and the recipient variety ACI Pakri-1 under both artificial and natural inoculums pressure. Slower rate of foliage destruction was observed in most of the selected resistant lines till 85 DAP since 1st infection. It may be due to host resistance of the selected lines, which reduced pathogen virulence as defined by decreased infection efficiency, diminished sporangia production, and a

reduction in the size of necrotic lesions (Black W, 1993). Eight lines were selected in LB resistant Category-1 (Fig. 4). Degree of sporulation recorded in most of the lines was 1 (Represents very sparse sporulation).

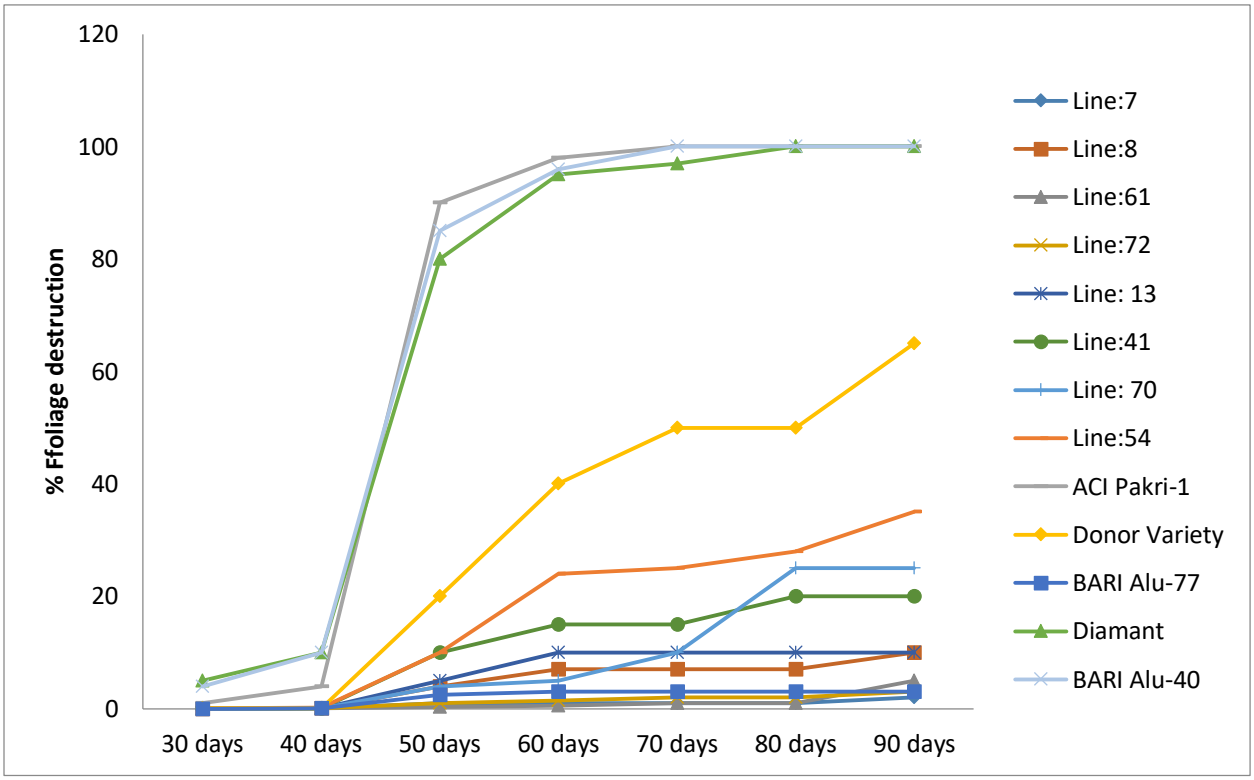

Fig.4. Foliage destruction due to LB in 8 selected resistant potato lines and parents

By using foliage destruction data, AUDPC was calculated, followed by relative AUDPC (rAUDPC) for 8 selected LB resistant lines along with check varieties. The value of AUDPC and rAUDPC represented the severity of disease. The AUDPC values ranged from 51.5 to 1047.5, and rAUDPC values ranged from 0.009 to 0.175 (Table 4). On the other hand, values of AUDPC and rAUDPC of parent materials were higher (ACI Pakri-1 = 4425 & Donor LB resistant variety = 1926.5) than the selected LB resistant lines.

Lines/ Varieties	AUDPC	rAUDPC	Susceptible scale value
Line:7	51.5	0.009	0.10
Line:8	301	0.050	0.61
Line:61	54.5	0.009	0.11
Line:72	81	0.014	0.16
Line: 13	401	0.067	0.82
Line:41	701.5	0.117	1.43
Line: 70	566	0.094	1.15
Line:54	1047.5	0.175	2.13
ACI Pakri-1	4425	0.738	9.00
Donor Variety	1926.5	0.321	3.92
BARI Alu-77	131	0.022	0.27
Diamant	4345	0.724	8.84
Bari Alu-40	4430	0.738	9.00

Table 4. AUDPC, rAUDPC and Susceptible Scale Value of 8 selected LB resistant lines and check varieties

Some lines had even lower AUDPC and rAUDPC than the resistant check variety BARI Alu-77, indicating that the lines are resistant to LB disease. Low rAUDPC values indicate low levels of infection during the evaluation period, corresponding to more resistant genotypes (Pérez W., 2008) However, susceptible scale value for 8 selected LB resistant lines and parent materials and check varieties were intended by using rAUDPC value. The highest susceptible scale value 9, was set by allowing for highest value of rAUDPC, 0.738 in BARI Alu-40 and ACI Pakri-1. The range of

susceptible scale value in selected LB resistant lines was 0.10 to 2.13, where donor variety had the value 3.92.

On the other hand, in total 14 lines were selected in the LB tolerant category (Category-2), which had medium level of foliage destruction by LB (Table 5). Among the selected 14 lines the foliage destruction due to LB ranged between 25 to 50 % at 85 DAP, which was lower than in the donor variety in both artificial and natural inoculums pressure. Higher degree of sporulation, 2 (representing sporulation on few leaves) to 3 (many sporangiophores being visible) was recorded in the lines of this Category than resistant lines.

Lines/ varieties	**Days to 1st lesion**		**Days to 80% or more infected plants**		**% Foliage destruction at 85 DAP**		**Degree of sporulation (0-3 scale)**	
	T^1	T^2	T^1	T^2	T^1	T^2	T^1	T^2
Cross-derived selected LB resistant lines								
Line:7	33	30	34	32	1	1	1	1
Line:8	35	34	37	35	10	7	1	1
Line: 61	36	30	38	32	4	5	1	1
Line: 72	39	33	42	35	2	3	1	1
Line: 13	31	34	35	36	9	10	1	1
Line: 41	34	29	36	32	19	20	2	2
Line: 70	38	41	42	46	26	25	2	2
Line: 54	30	30	32	34	28	25	2	2
Cross-derived selected LB tolerant lines								
Line:1	31	30	33	33	25	35	2	2
Line:2	37	38	40	40	25	30	2	2

Line: 6	30	30	34	32	25	25	2	2
Line: 11	34	33	36	34	50	53	3	3
Line: 18	29	29	31	30	40	45	3	3
Line: 32	31	33	33	35	40	40	2	2
Line: 44	34	32	37	35	25	25	2	2
Line: 40	35	34	38	36	35	30	2	2
Line: 53	34	30	39	33	50	40	2	2
Line: 68	33	32	35	35	25	25	2	2
Line: 71	40	38	44	44	45	30	2	2
Line: 29	33	33	35	36	48	43	3	3
Line: 56	33	33	34	35	40	45	3	3
Line: 45	35	34	37	35	50	40	3	3
Parents/ varieties (Check)								
ACI Pakri-1	29	28	29	29	100*	100*	3	3
Donor	30	30	32	34	65	58	3	3
Variety								
BARI Alu-77	41	38	45	47	2	3	1	1
Diamant	33	30	33	31	100**	100**	3	3
BARI Alu-40	29	29	31	31	100***	100***	3	3

Table 5. Infection, foliage destruction and sporulation in selected lines

Lastly, another 14 lines were selected in Category-3 (LB susceptible). These lines were selected on the basis of tuber color, shape, uniformity and yield. The lines were susceptible to late blight. The percentage of foliage destruction at 85 days was more than 50% to 90%.

3.2 Late blight gene confirmation:

Five gene specific primers were selected to find whether the late blight resistant genes were present or absent in selected 8 LB resistant lines. PCR product for Rpi-abpt amplified using marker R2-F1/R2-R3 found presence of resistant gene in donor variety as well as in the five lines, line 8, line 13, line 41, line 72 and line 54 among the eight selected LB resistant lines. On the other hand absence of Rpi-abpt gene in recipient variety (ACI Pakri-1) was found. Again late blight resistant gene, Rpi-blb1, was found absent in recipient variety, ACI Pakri-1, but present in donor variety, when DNA amplified using 1521/518 marker. Presence of Rpi-blb1 late blight resistant gene was also shown in line 8, 13, line 41, line 61, line 72, line 54 and line 70. However from these findings it is revealed that late blight resistant gene has been successfully transferred to recipient variety from the donor in most of the selected LB resistant lines as shown in Fig 5. Rest of the other three late blight resistant genes, Rpi-ber1, Rpi-sto1 and Rpi-bt1 was found present in all lines as well as parental materials. Line 7 shown high level of resistance against late blight in field screening but none of these two resistant genes, Rpi-abpt and Rpi-blb1, were found in these two lines. Resistance in line 7 in field screening may cause due to the newly formed resistant gene or gene combination through hybridization among donor and recipient variety. More field screening and MAS will be needed to find precise findings. However, based on these results, we can conclude that the Rpi-abpt and Rpi-blb1 genes are responsible for the resistance to *P. infestans* in the donor variety and therefore some selected LB resistant lines.

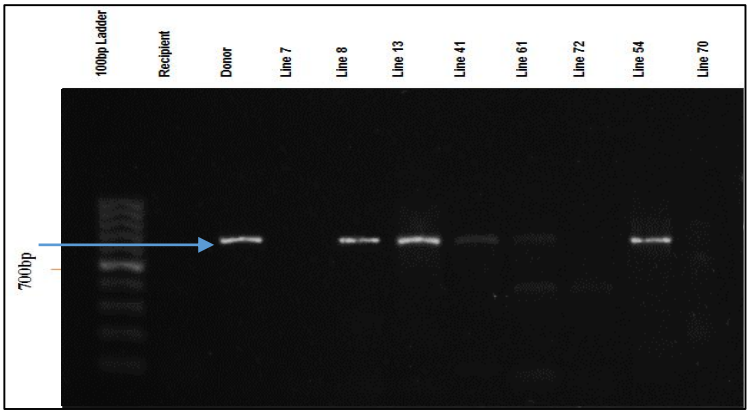

Figure 5: Confirmation of LB gene Rpi-abpt, using marker R2-F1/R2-R3, in the donor variety and in line 8, line 13, line 41, line 61 and line 54 respectively

Similar findings were found in the research of Chen *et al*, 2017 and reported that genotypic and phenotypic data was found correlate in case of late blight disease when DNA markers derived from the Rpi-blb1 gene was used for marker assisted selection.

3.3 Morphological features of tubers:

Tubers with deep to light red and white skin were found in LB resistant category (Fig.6). All tubers of cross-derived selected 8 lines were round shaped, as in the recipient parent ACI Pakri-1. In case of uniformity, uniform sized tubers were found in most lines as well as less uniform sized were observed in few lines. Eye color and eye depth of the selected lines were also recorded. Mostly red colored and deep in depth eyes were observed which was similar to parents.

Fig.6. Tubers of LB resistant category having different skin color.

Most of the lines under LB tolerant category had red to light red skinned and round tubers. Two lines having oval shaped tubers and one line with oblong shaped tubers were also found. On the other hand, mostly light red to red skinned tubers, and 3 white skinned tubers were found in Category-3 (LB susceptible, but with attractive tubers and high yield)**.** All round, but 1 oval shaped tuber line was recorded; and commonly, deep eye with red color was observed**.** Uniformity was at

satisfactory level**.** The morphological characteristics of the tubers of selected lines are presented in Table 6.

Lines/ Varieties	**Skin color**	**Shape of tuber**	**Color of eye**	**Depth of eye**	**Tuber uniformity (1-5 scale)**
Cross derived selected LB resistant lines					
Line:7	White	Round	Red	Shallow	5
Line:8	Red	Round	Red	Deep	4
Line: 61	White (Slightly red)	Round	White	Deep	4
Line: 72	Red	Round	Red	Deep	4
Line: 13	Light red	Round	Red	Deep	3
Line: 41	White (Slightly red)	Round	White	Shallow	4
Line: 70	Deep Red	Round	Red	Deep	3
Line: 54	White	Round	White	Shallow	5
Cross-derived selected LB tolerant lines					
Line:1	Light red	Round	Red	Deep	4
Line:2	Red	Round	Red	Deep	4
Line: 6	Light red	Round	Red	Deep	4
Line: 11	Red	Round	Red	Shallow	3
Line: 18	Light red	Round	Red	Deep	2
Line: 32	Light red	Round	Red	Deep	4
Line: 44	Light red	Round	Red	Shallow	4
Line: 40	Light red	Round	Red	Shallow	3

Line: 53	Light red	Round	Red	Deep	3
Line: 68	White	Oval	White	Shallow	4
Line: 71	White	Oblong	Red	Shallow	3
Line: 29	Light red	Oval	Red	Shallow	4
Line: 56	Red	Round	Red	Shallow	4
Line: 45	White (slightly red)	Round	Red	Shallow	4
Parents/ varieties (Check)					
ACI Pakri-1	Red	Round	Red	Deep	4
Donor Variety	Red	Round	Red	Deep	4
BARI Alu-77	Red	Long	Red	Shallow	4
Diamant	White	Long	White	Shallow	3
BARI Alu-40	White	Long	White	Shallow	4

Table 6. Morphological characteristics of the tubers of selected lines

3.4 Yield component and yield:

Data on yield component and yield were recorded at harvest after 90 DAP. Although there was wide variation in number of tubers per hill among the selected LB resistant lines (Category-1), the number was not significantly affected by the LB inoculums treatments (Table 4). But the weight of tuber per hill and estimated yield of tubers per hectare were noticeably influenced by LB inoculums treatment. The weight of tubers per hill ranged between 120 and 460 g among the selected 8 LB resistant lines under the no LB inoculums & no fungicide treatment. Application of LB inoculums did not show any positive response to yield of tubers per hill. Application of

fungicide was effective on selected lines. The weight of tubers ranged between 120-420 g/hill under the inoculation & no fungicide treatment, 120-460 g/hill under the no inoculation & no fungicide treatment, and 240-480 g/hill under the no inoculation & fungicide treatment. The corresponding estimated yield of tubers per unit area ranged between 10.0 -35.0, 10.0 - 38.3 and 20.0 - 40.0 Mt/ha, respectively (Table 7).

In case of the recipient variety ACI Pakri-1, the weight of tubers was only 31 g/hill when no inoculums or fungicide was applied, and only 20 g/hill when only inoculums was applied. But the weight of tubers was 348 g/hill when fungicide was applied. The weight of tubers per hill of the LB resistant check variety BARI Alu-77 was significantly higher under all levels of inoculums treatment, demonstrating 540 g/hill under fungicide application and 427 g/hill under no fungicide & no inoculums treatment. Here also, inoculums did not show any significant positive response. The LB susceptible check variety Diamant demonstrated weight of tubers per hill similar to ACI Pakri-1 as influenced by treatments receiving only inoculums or no inoculums & no fungicide. Among all treatments, the check variety BARI Alu-40 gave the highest yield of tubers/hill (610 g) when fungicide was applied.

As mentioned earlier, 14 potato lines were selected as LB tolerant materials (Category-2). The weight of tubers ranged between 60-300 g/hill under inoculation & no fungicide treatment, 60-300 g/hill under no inoculation & no fungicide treatment, and 280-460 g/hill under the no inoculation & fungicide treatment. Corresponding yields were, 5-25, 5-25 and 23-38 Mt/ha (Table 5).

Another set of 14 potato lines were selected as LB susceptible, but having attractive appearance of tuber and high yield (Category-3). In this category, the weight of tubers ranged between 17-45 g/hill under inoculation & no fungicide treatment, 20-47 g/hill under no inoculation & no fungicide

treatment, and 320-490 g/hill under the no inoculation & fungicide treatment. Corresponding yields were, 1.4-3.4, 1.7-3.9 and 26.7-40.8 Mt/ha.

It has been reported that, potato yield loss primarily due to late blight is dependent on variety susceptibility or tolerance/ resistance, and disease management practices (Chen S., 2017 & Dey TK., 1994)

The findings of the present study agree with the opinion of Kankwatst *et al.*,, who did mention that integration of host resistance and fungicide application reduced late blight severity by more than 50 %, and increased yield by more than 30%.

Although the number of tubers per hill was similar among the LB tolerance lines and parent materials, some lines showed significantly higher weight of tuber per hill than ACI Pakri-1. All LB tolerance lines showed higher yield per hectare than ACI Pakri-1 when treated with artificial inoculums pressure and without any fungicide application. The highest yield among LB tolerant lines was 25.0 Mt/ha (in Line: 1), when LB inoculation was done but no fungicide was applied. The yield of the line became 35.0 Mt/ha when fungicide was applied. Some research results indicated that, 50 to 70% yield loss might occur due to late blight, depending on degree of resistance of the cultivar (Doyle JJ., 1990 & Flor HH., 1971)

Lines/ Varieties	No. of tubers/hill			Wt. of tubers/hill (g)			Yield of tubers (Mt/ha)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
Cross derived selected LB resistant lines									
Line:7	17.4	16.0	18.6	380	360	400	31.7	30.0	33.3
Line:8	18.0	20.0	17.4	320	360	370	26.7	30.0	30.8
Line: 61	11.4	16.2	15.0	420	460	480	35.0	38.3	40.0
Line: 72	13.6	12.2	17.0	300	280	320	25.0	23.3	26.8
Line: 13	11.6	10.8	11.8	220	200	320	18.3	16.7	26.7
Line: 41	7.6	9.6	10.0	120	120	240	10.0	10.0	20.0
Line: 70	14.0	14.3	9.0	150	167	267	12.5	13.9	22.3
Line: 54	13.7	14.8	16.0	167	160	340	13.9	13.3	28.3
Cross-derived selected LB tolerant lines									
Line:1	13.4	14.8	16.2	300	180	420	25.0	15.0	35.0
Line:2	5.0	8.0	15.0	145	180	400	12.0	15.0	33.3
Line: 6	7.2	10.4	10.2	140	140	320	11.7	11.7	26.7
Line: 11	8.2	12.2	15.8	120	160	420	12.5	13.3	35.2
Line: 18	6.0	4.4	14.4	60	60	360	5.0	5.0	30.0
Line: 32	8.6	11.6	16.4	220	220	420	18.3	18.3	35.0
Line: 44	10.0	11.0	12.0	240	300	440	17.5	25.0	36.7
Line: 40	14	11	20	160	140	320	13.3	10.8	26.7
Line: 53	9.8	10.4	18.8	220	260	460	18.3	21.8	38.3
Line: 68	5.4	7.6	13.0	160	260	380	13.3	21.8	31.7
Line: 71	4.6	6.6	9.2	140	260	400	11.7	21.7	33.3
Line: 29	9.6	7.8	16.6	120	160	360	10.0	13.3	30.0
Line: 56	6.6	7.2	18	70	80	400	5.8	6.7	33.3

Line: 45	6.2	8.0	9.0	100	120	280	8.3	10.0	23.3
Parents/ varieties (Check)									
ACI Pakri-1	6.0	9.6	29.8	20	31	348	1.7	2.6	29.0
Donor Variety	9.6	10.0	14.8	223	252	378	18.6	21.0	31.5
BARI Alu-77	9.6	8.0	9.8	468	427	540	39.0	35.6	45.0
Diamant	4.4	3.0	7.8	27	47	322	2.3	3.9	26.8
Bari Alu-40	10	10.4	14	156	96	610	13.0	8.0	50.8

Table 7. Yield components and yield of selected lines

The lines under Category-3 were selected not on the basis of their resistance against LB, but on the basis of attractive appearance of tubers and high yield. Among the selected 14 susceptible lines, the highest number of tubers per hill was found in Line-17. Some lines had higher weight of tubers per hill than the parent materials. The estimated yields were 40.8 Mt/ha in Line-35 and Line-36, and 39.7 Mt/ha in Line-65.

3.5 Conclusion:

The present experiment was conducted in one season for screening of hybrid-derived potato materials against LB. Preliminary LB resistant and tolerant lines were found but it would not be reasonable to make concrete suggestion and recommendation for late blight resistant potato variety. However, this study could be helpful for potato resistant breeding especially in screening methods of potato germplasm/lines against LB. Further screening against late blight under late blight inoculums pressure is suggested. Finally, based on the potentiality against LB and yield performance, Line 8 was registered as potato variety namely ACI LBR Pakri-1 by Seed Wing, Ministry of Agriculture, Bangladesh.

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