# Determining the Type of PVY Extreme Resistance Genes ($Ry_{adg}$ and/or $Ry_{sto}$) in Genotypes Used in the Breeding Programs of the Potato Research Centre

**Mr. Molla Gereme Taye**

Department of Plant Biotechnology, Tigray Agricultural Research Institute, Mekelle, Ethiopia

***Abstract-*** The potato virus Y (PVY) is disseminated globally and is one of the most devastating viruses with respect to yield and quality deteriorations in the potato (*Solanum tuberosum* L.). PVY caused crop losses may reach even 80%. The use of extreme resistant cultivars is assumed to be the most effective and sustainable long term way of reducing crop losses associated with PVY virus. To cut down this impingement, there is an immense interest in obtaining genotypes carrying the $Ry_{adg}$ and $Ry_{sto}$ genes that provide extreme resistance to this virus. These two genes derived from *Solanum tuberosumssp S. andigena* as well as *S. stoloniferum*, respectively, are reside on different chromosomes. Introgression of these genes could be facilitated using the $Ry_{adg}$ specific RYSC3 and the $Ry_{sto}$ specific ST1 SCAR (Sequence Characterized Amplified Region) markers for marker - assisted selection (MAS). The objective of the present study was to evaluate different potato genotypes with these SCAR markers for the presence or absence of $Ry_{adg}$ and /or $Ry_{sto}$ PVY extreme resistance genes. Of a total of 13 evaluated potato breeding lines three genotypes (Cmm16, 96.367 and FH97. 029. 02) revealed resistance to the virus (PVY), and it was found that they are carrying both the RYSC3 and ST1 markers; whereas the RYSC3 marker could be detected in all tested genotypes. Here, we declared this highly effective (100%) detection of the $Ry_{adg}$ gene confirmed that the marker is appropriate in MAS of potato genotypes with extreme PVY resistance. Our results indicate that marker assisted selection for these two genes could be effectively used in potato breeding for the production of PVY extreme resistance genotypes, even in hetero-multiplex condition.

***Index Terms***- Extreme resistance: MAS: PVY: $Ry_{adg}$: $Ry_{sto}$: *Solanum tuberosum*

## I. INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most prominent world crop ranked third, next to rice and wheat (FAO, 2013). Furthermore, it is one of the well-known root and tuber crops as globe's number one non-grain food commodity (FAOSTAT, 2008). Once harvested, potatoes are utilized as fresh food, for processed food products and food ingredients, animals feed, biofuel, and as seed tubers. In addition, it is a source of carbohydrates, and also contains high content of protein, vitamin C, fiber, and minerals and has a low fat content; recently it becomesone ofthe principal crops that have a Lion share contribution in physical and nutritional food security almost all over the world (Varvarre*et al*., 2009). Due to the globe wide importance of potatoes for various reasons, including agriculture, economy and world food security, the FAO declared 2008 the "International Year of the Potato", where the main aims were to promote the sustainable development of potato-based systems and the potato industry and to enhance the well-being of producers as well as consumers (FAO, 2008).Despite the fact,the fresh -weight yields of potatoes has been varied enormously by country from 2 to 50 tons ha-1. Specifically, in 2007 production season about 300 million tons of fresh-weight tuber yields has been obtained from nearly 18 million hectares of cultivated lands of potato with a global average of 16.7 tons ha-1 (http://faostat.fao.org). Of which the European share was approximately 40% (FAO, 2004a). However, China is the world's largest potato producer. On the other hand, potato consumption in developing countries has increased during recent years but it is still at a low level in comparison with consumption in Europe (94.2 kg/capita/year) in 2001 (FAO, 2004b). Recently, potato's gross production and total cultivated areas has been exhibiting slight progress globally. For instance, in 2010 it was nearly 324 million tons 19 million ha-1 of land approximately (FAOSTAT, 2011). Invariable, a rapid potato tuber yield increment has also been reported by (Fuglie, 2007; Harverkot*et al*., 2009) particularly in Africa. Although, the yield paradox still goes on, as the unevenness of potato tuber yields and quality deteriorations from region to region remains nearly great due to biotic and abiotic stresses.

Potato virus Y (PVY) was first recognized in potato in 1931(Smith, 1931) as a member of pathogens associated with potato degeneration, a disorder known since the 18th century. It is one of the most economically important viral pathogen of *solanaceous* crops, which belongs to the genus Potyvirus (family Potyviridae), is the largest group of plant viruses, and thought to be one of the most destructive families of plant viruses affecting potato crops utmost (Shukla *et al*., 1994). Consequently the virus (PVY) causes serious losses in both potato´s yield and quality (80%, De Bokx&Hittinga, 1981). Although the severity of the disease and the substantial losses of produces are dependent on PVY strain involved, host tolerance, time of infection and environmental factors. The genome of PVY is composed of 9704 nucleotides (Robaglia*et al*., 1989) as well as potato virus A (PVA) and potato virus V (PVV). Similarly the report of (Dougherty &Carrigton, 1988) has also shown that as the viral genome of PVY comprises of a single-stranded, positive-sense RNA molecule of nearly 10 kb in length. There are three main strains of this virus: $PVY^O$ (common strain), $PVY^N$ (tobacco veinal necrosis strain), and $PVY^C$ (stipple-streak strain, including

potato virus C) (Brunt, 2001). Additional strains have also been documented including $PVY^{-NTN}$, responsible for the potato tuber necrotic ring spot disease, $PVY^{NW}$, and $PVY^{N:\ O}$ (Kerlan*et al*., 1999). Due to PVY infection symptoms which include vein necrosis, mottling, yellowing of leaflets, leaf-dropping, plant dwarfing and premature plant death will be recognized (De Bokx&Hittinga, 1981), but differences in symptom expression can be highly cultivar and virus strain specific. Some of the main diseases caused by PVY comprehend mild to severe leaf molting, streak or ´leaf-drop streak´ ($PVY^{O}$) with necrosis along the veins of the underside leaflets ($PVY^{N}$) and ´stipple-streak´ ($PVY^{C}$) (Harison, 1984).

The virus also infects several crop species in the family *Solanaceae* (tomato, pepper & tobacco) (Brunt et al., 1996). Furthermore, it can also cause disease in some members of the families Chenopodiaceae and Leguminosae, so it remains as one of the viral pathogen comprising wide range host. In general virus infections give rise to serious problems in vegetative propagated crops like potato because of virus manifestation through all subsequent vegetative generations.The distribution of PVY is global, although some virus strains are restricted to certain continents (Harison, 1984). $PVY^{O}$ strains occur worldwide, $PVY^{N}$ strains occur in Europe, parts of Africa & South America, and $PVY^{C}$ strains have been reported from Australia, Asia and Europe. In potato, the PVY virus can be transmitted by sap inoculation, grafting and aphids in a non-persistent manner (Sigvald, 1984). Even though PVY can also be spread mechanically, this mode of transmission is usually of minor importance in the field. Non-persistence viruses have a loose association with vectors, i.e. virus specificity is not marked and many vectors can transmit the virus. In the case of PVY, aphids are the most important transmitting vector. More than 50 aphid species have been shown to be able to act as vectors of PVY, although some species are more efficient than others (Radcliffe & Ragsdale, 2000). For instance, the most efficient vector appears to be the cosmopolitan peach-potato aphid Myzuspersicae (Hemiptera: Aphididae) (De Bokx&Hittinga, 1981), which also known as the green peach or peach-potato aphid.

Due to the above heated issues, the use of PVY resistant cultivars is became an alternative or complementary control method. This option is environmentally friendly, cost-effective and easy to implement by the growers since the solution is to plant resistant varieties. PVY-resistant cultivars can be obtained through genetic transformation (Missiou*et al*., 2004). However, molecular breeding is the current means of developing PVY resistant cultivars due to the non-acceptance of transgenic varieties by the potato industry and consumers. This breeding scheme for resistance to PVY starts with the identification of potato clones resistant to PVY, followed by the introgression of the resistant gene(s) into advanced breeding populations, to ensure this; primarily the utility of new tool is required to assist the screening and selection of new genotypes (Tankesly*et al*., 1989). The first resistance mechanism can be characterized by the lacking of infection (immunity) or by strong reduction of virus replication in infected cells. While the second resistance mechanism inhibits virus spread from cell-to-cell and through the vascular system (Bendahmane*et al*., 1999). The hypersensitivity reaction inhibits virus movement and belongs to the second group of resistance but does not provide a total defense against virus Y. Particularly the two major types of mono-genetically inherited resistance genes to PVY are known in cultivated and wild potato species (Solanum spp.), namely hypersensitive resistance (Ny) and extreme resistance (Ry) also designated as immunity (Ross, 1986). Thus, the use of extreme resistance cultivars is considered to be the most effective and sustainable long term way of minimizing crop losses associated with PVY or other viruses.Therefore, our present study was aimed at the screening of different potato genotypes (13) using SCAR (Sequence Characterize Amplified Regions) molecular markers to detect the presence or absence of the $Ry_{adg}$ and $Ry_{sto}$, PVY extreme resistance genes.

## II. MATERIALS AND METHODS

### 1.1. Plant Material

Thirteen breeding lines or genotypes evaluated for the presence or absence of molecular markers were chosen from the potato gene bank of the Potato Research Centre (University of Pannonia, Keszthely, Hungary). Thus usedgenotypes are shown in (Table 2). White Lady and W1100 were used as control in evaluating the genotypes for the presence and absence of extreme resistance genes respectively. All the plant materials used in this study were grown in the farm of the Potato Research Centre, Keszthely in extensive agriculture, and the leaf sample of each and every test material was also taken in the early growth stage by non-destructive method of sampling. Subsequently those sampled leaves were kept in cold storage at-20$^0$C in the presence of liquid nitrogen till DNA extraction proceeds.

### 1.2. DNA Extraction and Polymerase Chain Reaction

In the molecular based screening for PVY extreme resistance of the various genotypes, the total genomic DNA was obtained from the leaves of field grown potato plants, according to Walbot& Warren method (Walbot& Warren, 1988) with minor modifications. The leaf samples were collected, cut in to pieces, placed in Qiagen collection tubes and stored at-20$^0$C together with liquid nitrogen until DNA extraction. The genomic DNA was extracted from 20mg of young leaf tissue. The measured weight of leaf was put into the Eppendorf tube and ground mechanically using minimal quartz sand and pestle in the presence of liquid nitrogen. The grounded leaves were conveyed to 1.5ml Eppendorf tube and 1.3 ml lysis buffer (15% sucrose, 50mM Tris (pH8.0), 50mM EDTA (Ph8.0) and 500mM NaCl was added. Subsequently up on centrifugation (5 min, 4$^0$C, and 6000 rpm) the fluid phase was discarded. Three hundred micro liters of 20T-10E buffer (20mM Tris-HCl, 10mM EDTA) and 20µl 20% SDS were added to the pellet. Samples were shacked and vortexed thoroughly, then incubated at 70$^0$C for 15 min.

Potassium- acetate was added to the sample (150µl, 7.5M) the mixture was left for at least 30 min. After centrifugation (10 min, 4$^0$C & 14500 rpm), the supernatant was carefully displaced to the new tubes (400-450µl) containing 500µl isopropanol. Samples were also incubated at room temperature for 15 min. Then after precipitation and centrifugation, the liquid was spilled out of the tubes, and the pellet was re-suspended in TE buffer (500µl). Next CIA (Chloroform isoamly alcohol 25:1) treatment,

samples were centrifuged at room temperature (5 min & 800 rpm). The upper phase was carefully pipetted out, and then the pellet washed with 99.5%, followed by centrifugation (10 min, $4^0$C & 14500 rpm). At the end of this particular step the liquid phase was discarded, while the pellet was resolved in 400µl 70% ethanol. Eventually, the dried pellet (DNA) was re-suspended in 200 TE buffer and stored at-$20^0$C in the presence of liquid nitrogen until the successive molecular procedures would be run.
PVY extreme resistance $Ry_{adg}$ and$Ry_{sto}$ genes were subjected for detection utilizing the RYSC3 and ST1 SCAR markers after PCR amplification. These markers are closely connected to and pointed out a high degree of precision for the detection of the $Ry_{adg}$ and $Ry_{sto}$ genes. In this investigation polymerase chain reaction (PCR) amplification of markers proximate to $Ry_{adg}$ and $Ry_{sto}$ were carried out using thermo cycler with primers explicated by (Kasai *et al*., 2000) and Z. Bánfalvi (ABC, Godollo, Hungay ) respectively. For the magnification of a segment of $Ry_{adg}$ gene; we used a pair of primer sets: Primers to amplify RYSC3 marker with PCR were described by Kasai et al. (2000) and coincide with the oligonucleotides 3.3.3s (5´-ATA CAC TCA TCT AAA TTT GAT GG-3´) and ADG23R (5´-AGG ATA TAC GGC ATC ATT TTT CCGA-3´). The reaction mixture was comprised of, 2Mm MgCL2, 0.125 mM dNTP, 0.25µM each primer, 1unit of Taq DNA polymerase, 50µg of DNA template and ultra-pure water were used to attain a final reaction volume of 20µl. The PCR program for the RYSC3 marker consisted of a first denaturation step at $94^0$C for 2min, followed by 35 cycles of denaturation at $94^0$C for 15 sec, primer annealing at $55^0$C for 15 sec, extension at $72^0$C for 40 sec, with a final extension at $72^0$C for 5 min, and stored at $4^0$C.

For the ST1 marker unpublished primers obtained by courtesy from Z. Bánfalvi (ABC, Godollo, Hungary). PCR was conducted in a final reaction volume of 12µl. PCR for the in vitro replication of ST1 unpublished marker was consisted of the proceeding reaction mixture components: 50ng template DNA, 2mM dNTP (dATP, dTTP, dCTP, dGTP), 2Mm x 10 PCR buffer {(1x 10mM TrisHCl, (Ph8.8, 1.5mM MgCl2, 50mM KCl, & 0.1% Triton X- 100)}, 10pM each of the primers (Sigma Genosys Ltd.) and 0.4 unit of DNA polymerase enzyme were used, and accomplished in Eppendorf tubes Master cycler /thermo cycler. The artificial replication profile embraced preliminary denaturation for 3 sec at $94^0$C, followed by 35 cycles of denaturation at $94^0$C for 1 min, primer annealing at $58^0$C for 1 min, extension at $72^0$C for 1 min, final synthesis at $72^0$C for 7 min, and then stored at $4^0$C.

### 1.3. Gel electrophoresis and documentation

Once the predetermined number of cycles (35) ran, then the amplicons of PCR products were subjected to electroporation in a 1.5% agarose gel (Promega, USA) in 0.5X TBE (pH 8.0) for 1:30 hrs. Loading dye was used to make easy DNA band tracking within the gel during size fractionation, besides adding weight to the samples which in turn helped the bands to settle down to the bottom of the wells. Afterward the amplified bands were dyed in a 5µl mL-1 ethidium bromide solution for 20 min, were also visualized and photographed with a digital photo camera in a UV light Trans illuminator and documented with a GenGenuis Bio Imaging System (Syengene, UK).

### 1.4. Detection of the presence or absence of extreme resistance gene (s)

Genotype resistance or susceptible to PVY virus was determined based on the presence or absence of $Ry_{adg}$ and $Ry_{sto}$ extreme resistance genes after PCR amplification and UV light visualization of specific markers. The presence of bands similar to positive control and the absence of bands in the case of negative control revealed and were corresponded with the presence and absence of extreme resistance or susceptible genes accordingly. This approach coupled with expected marker band size was used for the screening of the potato genotypes associated with PVY extreme resistance in our investigation. Specifically, presence of assumed marker band sizes (321-bp) for RYSC3 and ST1 (293-bp) were a companioned with the existence of PVY extreme resistance genes ($Ry_{adg}$ and $Ry_{sto}$) respectively, whereas, absence of the amplified products and formation of monomorphic band patterns are associated with the absence and /or failure of marker to detect these extreme resistance genes $Ry_{adg}$ and $Ry_{sto}$ accordingly (Kasai *et al*., 2000, Z. Bánfalvi).

## III. RESULTS

The breeding lines from the Potato Research Centre used in this experiment differ in genetic background. However, the protocol used for DNA extraction and the allele specific marker chosen for the analysis allowed a simple and fast method to know the Ry presence on the evaluated germplasms. The amplicons obtained were of the expected weight and the presence of markers and/or the banding patterns are shown in (Figure.1) which presents the successful molecular evaluation of the breeding lines. The cultivar White Lady, widely used for $Ry_{adg}$ and $Ry_{sto}$identification, showed not only the characteristic 321 bp band using the RYSC3 marker but also the ST1 marker (293 bp). In fact, (Hámáláinen*et al*., 1997; Kasai *et al*., 2000) reported the specificity of the RYSC3 marker revealing only $Ry_{adg}$in genotypes introgressed the extreme resistance from *S. tuberosum ssp. andigena*. The present study's result specifically for RYSC3 marker is agreed in most cases with the previous findings.

Most of the genotypes (10), which show ER to PVY, only the marker RYSC3 were detected; while the marker ST1 was absent (Table 2). The molecular phenotypes or banding patterns, observed for the ST1 marker in 13 breeding lines only 3 produced a 293 bp band size and appeared with polymorphic band configuration associated with resistance to PVY ($Ry_{sto}$, present), while the remaining 10 genotypes showed monomorphic bands, indicating the absence of the marker and also most likely the correspondence extreme resistance gene. Moreover, the RYSC3 marker was also detected in these three genotypes. The susceptible genotype, used as a negative control was able to show neither of the Ry gene markers. These results of PCR analysis for the presence of extreme resistance genes $Ry_{adg}$ and $Ry_{sto}$ were compared with White Lady and W1100, which are phenotypically resistance and susceptible sequentially. Table 2 delivers the core data of the examinations.

**Table 1 Markers used in the analysis**

| Gene | Marker | Presence of marker |
|---|---|---|
| $R_{Y\ adg}$ | RYSC3 | 13(13) |
| $R_{Y\ sto}$ | ST1 | 3(13) |

$Ry_{adg}$= PVY extreme resistance gene derived from *S.tuberosum ssp. andigena*
$Ry_{sto}$ = PVY extereme resistance gene gained from *S. stolonifereum*
Number of analyzed genotypes is in parenthesis

**Table2 Presence/absence of RYSC3 and/or ST1 markers of $Ry_{adg}$ and $Ry_{sto}$genes in potato germplasms of the Potato Research Centre, with their correspondence pedigree profile**

| No | Genotypes | RYSC3 | ST1 | Type of PVY resistance gene (s) | | Pedigree |
|---|---|---|---|---|---|---|
| 1 | 2 | + | -[a] | $Ry_{adg}$ | -[c] | TRN[1](BC1) |
| 2 | 7/27 | + | - | $Ry_{adg}$ | - | TRN[2](BC2) |
| 3 | Cmm16 | + | + | $Ry_{adg}$ | $Ry_{sto}$ | -[d] |
| 4 | 76. 9104 | + | - | $Ry_{adg}$ | - | spc.dem,and |
| 5 | 01.220 | + | - | $Ry_{adg}$ | - | 90.364x89.451 |
| 6 | 01.739 | + | - | $Ry_{adg}$ | - | Kastia x Concurrent |
| 7 | 89.45 | + | - | $Ry_{adg}$ | - | S.andigena |
| 8 | 96.367 | + | + | $Ry_{adg}$ | $Ry_{sto}$ | 86.143 x Kastia |
| 9 | 01.1395 | + | - | $Ry_{adg}$ | - | Darsow |
| 10 | 98.7804.01 | + | | $Ry_{adg}$ | - | Darsow |
| 11 | 94.7243.03 | + | - | $Ry_{adg}$ | - | Darsow |
| 12 | FH97.029.02 | + | + | $Ry_{adg}$ | $Ry_{sto}$ | Darsow |
| 13 | W231 | + | - | $Ry_{adg}$ | - | Wisconsin |
| 14 | White Lady | + | + | $Ry_{adg}$ | $Ry_{sto}$ | PRC |
| 15 | W1100 | - | - | -[b] | - | Wisconsin |

**$Ry_{adg}$**=Extreme resistance derived from *S. tuberosum ssp. andigena*, **$Ry_{sto}$**=Extreme resistance gene derived from *S. stoloniferum* ´+´ = Presence of marker, **–[a]**= Absence of marker, **–[b]**=Absence of the $Ry_{adg}$,**–[c]**=Absence of the $Ry_{sto}$, **–[d]**= Lack of the pedigree data, **and**= Solanum andigena, **BC1**= First back cross generation, **BC2**= Second back cross generation, **dem**= Solanum demissum, **spe**= Solanum spectabile, **TRN**= Solanum tarnii

The RYSC3 marker was used to determine the presence of the $Ry_{adg}$ extreme resistance gene over a population of 13 genotypes of the Potato Research Centre Germplasm, corresponding to the breeding lines used (Table 2). The result of analysis showed that all the 13 genotypes (100%) were carrier of the marker excluding (W1100) the negative control (Table 2). No genotypes are screened and recorded as susceptible or did not show up a product, in the presence $Ry_{adg}$ gene test. Despite the existence of various theoretical and practical factors that might lead to false positive or negative results as mentioned above, but in this particular study since the negative control did not generate a product and supported by the generation of the expected marker band size correlated with the phenotypic resistance variety (White Lady). So the detection is said to be unbiased by the potential threats rather it could be due to the marker´s high efficiency in detecting $Ry_{adg}$ marker specific extreme resistance gene, as it is also reported by (Boris *et al*., 2008) with 99.7% effectiveness. The PCR amplification which shows the presence or absence of the target marker which in turn confirms the existence or nonappearance of the correspondence extreme resistance gene depicted in (Figure.1).

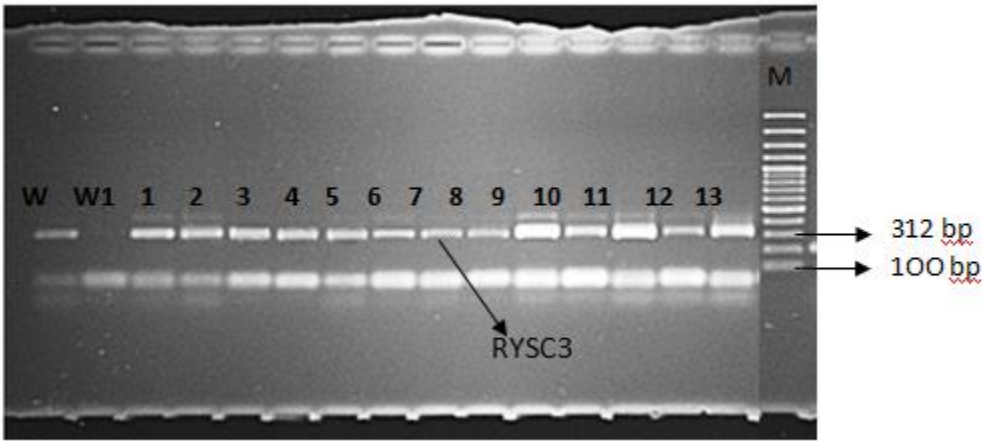


Figure.1: Gel photo depicting the polymerase chain reaction (PCR) amplification products with RYSC3 marker. Presence of the marker or 321 bp products indicate the presence of the Ryadg gene. Absence of the marker (the marker band) shows the absence of the extreme resistance gene. W= PVY resistance variety (White Lady, positive control), W1= PVY susceptible genotype (W1100, Negative control), M= Molecular size marker 100 bp DNA ladder. Lanes 1-13= represent the investigated breeding lines.

The ST1 marker was used to detect the existence of $Ry_{sto}$ extreme resistance gene across 13 breeding lines of the Potato Research Centre germeplasms listed in (Table 2). The outcomes of the analysis revealed that 3 of 13 genotypes are carrying the ST1 marker. These genotypes (Cmm16, 96. 376 and FH97.029. 02) also contained the PVY extreme resistance $Ry_{adg}$ gene. Despite the closer genetic distance of the marker to the gene of interest (1.3cM), its detection efficiency was low. This would be mainly due to the presence of a different allele, the existence of $Ry_{adg}$ gene, and /or the influence of genetic distance between the gene and marker. Figrue.2 shows the results for the ST1 marker analysis and the occurrence of the marker delineated as the existence of the gene (polymorphic bands configuration).

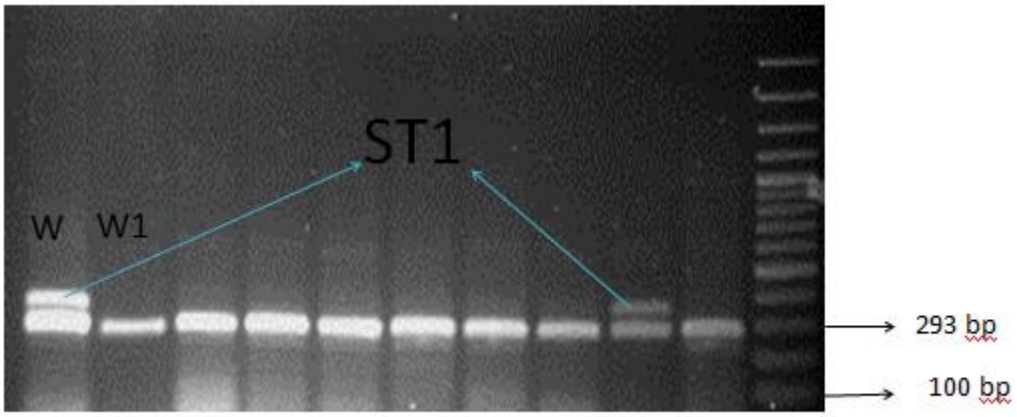


Figure.2: Gel photo illustrating polymerase chain reaction (PCR) amplification products with ST1 marker. Presence of the marker (=/or polymorphic banding patterns) shows the existence of the PVY extreme resistance gene (Rysto). Absence of the marker (−/or monomorphic banding patterns) indicates the nonappearance of the extreme resistance gene. M= Molecular size marker 100 bp DNA ladder. W= PVY resistance variety (White Lady, Positive control). W1= PVY susceptible genotype (W1100, Negative control).

## IV. DISCUSSION

As described in the literature, currently MAS in plant breeding is becoming more economically feasible since various diagnostic marker techniques are developed and used. Consequently shifts to marker-assisted selection became the most appropriate complementary means of crop improvement. From 1990 till recent, over forty major qualitative extreme resistance genes have been genetically mapped. These genes have been confirming a prominent protection against pathogens and insect pests affecting potatoes. However, the validation and down earth application of those findings in terms of MAS in potato crop amelioration programs has been creeping. Issued examples of utilization of molecular markers for MAS of pests are mainly resistance restricted to diploid material and minute number of genes i.e. Ns for PVS resistance (Macrczewski*et al*., 2006); $Ry_{adg}$ extreme resistance to PVY, Grol for resistance to *G. rostochiensis*, Rx1 extreme resistance to PVX or Senl for resistance potato wart (Gebhardt*et al*., 2006). Furthermore, the ST1 unpublished molecular marker is already used in the breeding programs of the Potato Research Centre. Scholars acceded, as more resistance loci are marked, it is assumed that

the application of MAS in the development of incipient potato cultivar will accelerate in supremacy. Molecular based screening of breeding lines is possible whenever there is a tissue from any component of the crop for DNA isolation and successive PCR assays, and then the acquired results from MAS facilitates classification and selection as resistance or susceptible for PVY virus. Degree of successfulness of molecular based evaluation for distinguishing characters of interest will be influenced by the genetic position between the marker in use and the gene of interest.

In the present study thirteen genotypes (2, 7/27, Cmm16, 76. 9104, 01. 220, 01.739, 89. 45, 96. 367, 01.1395, 98. 7804, 94.7243.03, FH97.029.02 and W231) were grouped and subjected to molecular evaluation for the presence of PVY extreme resistance gene $Ry_{adg}$ using the RYSC3 maker developed by (Kasai et al., 2000). Similarly, these genotypes were also investigated for the presence of the PVY extreme resistance gene ($Ry_{sto}$) by utilizing the unpublished ST1 marker. The pedigree data were available for all candidate genotypes except for Cmm16 were missed. However, both of the RYSC3 and ST1 markers used were effective in detecting three individuals (Cmm16, 96. 376 and FH97.029.02 ) carried PVY extreme resistance genes $Ry_{adg}$ and$Ry_{sto}$ in common. This could be elucidated by the genetic distance between the gene and the marker and /or by the presence of different alleles, besides it is tangible that they owned another source of resistance ($Ry_{adg}$) according to the results of this study. Moreover, in the analysis of ST1 marker, breeding lines, which did not show positive signals, could be due to their distant relationship between *S. stoloniferum* or because of unsuccessful intercrossing.

On the other hand, the RYSC3 maker was capable to detect all the tested genotypes as conveyer of PVY extreme resistance gene $Ry_{adg}$ derived from *S. tuberosum ssp. andigena*. The primers were said to be effective in amplifying the expected marker band size (321-bp) which coincides with the finding of (Kasai *et al*., 2000) the developer of the RYSC3 marker utilizing identical primers (3. 3.3 s & ADG23R), and explained that the presence of such band size associated with PVY extreme resistance from $Ry_{adg}$ while absence indicated the susceptibility. This could be due to their close genetic relation with *Solanum tuberosum ssp. andigena* and/or compatibly intercrossed, besides the specificity of the marker. Hence, the pedigree data of extreme resistance parental lines should be verified for the presence of extreme resistance gene(s)/ markers a head of evaluation using markers.

The $Ry_{adg}$ gene has been shown to be non-strain specific providing durable resistance to ample sources (Michovilovich*et al*., 1998), However, the efficacy of a marker like RYSC3 to trace carrier progenies of the resistance gene $Ry_{adg}$ to PVY can be negatively mannered by sundry causes such as inadequate PCR conditions, exorbitant distance between the marker and the gene loci that lead to its recombination, presence of other alleles of the same locus not aforetime identified and/or coalescence of the PCR primers in other sequences of the genome that amplify products of homogeneous size that do not correspond to the marker. Indiscriminative PCR conditions with low alignment temperature and/or high primer concentration and Mg+2 can produce unspecified PCR products. From one standpoint, very rigorous PCR conditions can minify amplification efficiency of the product. Both situations could lead to an error with an incremental of the number of mendacious positives or negatives, respectively. Owing to the fact that PCR conditions can vary between laboratories, marker reproducibility was valuated under our work conditions. The ability to check for the presence of $Ry_{adg}$ and $Ry_{sto}$ for hybridizing parents a head of the field screening of progeny can enhance selection efficiency for PVY resistance. This data reveals a clear harmony between the markers of *S. tuberosum ssp. andigena* and *S. stoloniferum* derived populations from a cross of the PVY resistance variety (White Lady) and PVY susceptible genotype (W1100). When all two tested markers were present in the variety White Lady, while it was evident that those markers were not able to be detected in the genotype W1100.

The RYSC3 marker was acclimated to determine the presence of the $Ry_{adg}$ gene over a population of 13 genotypes of the Potato Research Centre germplasms, corresponding to the progenitors frequently utilized (Table 2) shows the presence or absence of the RYSC3 marker for the tested genotypes. Specifically the result of RYSC3 marker analysis reveals that all the targeted individuals (100%) of the germplasms resistance to PVY were carrier of the RYSC3. The negative control (W1100) did not engender any product. Therefore, it could be verbally expressed that in these genotypes, the marker was highly efficacious, which is 100%, the identification of individuals resistance to PVY carriers of the $Ry_{adg}$ gene. According to (Dalla Rizza *et al*., 2006) the utilization of *S. tuberosum ssp. andigena* (RYSC3) and*S. stoloniferum* (M45) markers in a breeding program has recently been suggested. This would be because of their finding manifested highest efficiency approximately 99% coupled with the results show concurrency with the RYSC3 and M45 markers in 46 of 47 clones tested. Likewise, Jonathan et al., (2009) reported that $Ry_{adg}$ mediated resistance confers PVY resistance against all strains besides proximately 100% detection efficiency. Furthermore, Kasai et al. (2000) suggested that the SCAR markers (RYSC3 & RYSC4) might additionally subsidiary in detecting PVY resistance, because of the tight linkage (6.8cM) to the $Ry_{adg}$ gene (Hámáláinen*et al*., 1997;Kasai *et al*., 2000). However, Knowledge of parental background of a clone is paramount for, the utilization of markers that can detect when resistance gene(s) are genuinely present. According to Ottoman *et al*. (2009) nesting/pyramiding of several genes for resistance PVY (for instance, $Ry_{adg}$ and $Ry_{sto}$) in a parental line would also develop PVY resistance clones. Marker -assisted selection would be the only destine for tracking the presence of major genes confirming extreme resistance to PVY.

## V. CONCLUSION

In summary, of a total of 13 genotypes from the Potato Research Centre, all were identified as carrying extreme resistance gene (s) due to the presence of Ry gene(s) trace in this study. All the evaluated genotypes are detected as the carrier of the RYSC3 marker which was 100% efficient in this particular study. Based on the outcome of the analysis, only three genotypes contained both markers in common. For the genotypes tested in this experiment, the RYSC3 marker proved to be highly useful for tracing $Ry_{adg}$ and $Ry_{sto}$ genes. Thus, this molecular approach proved to be very effective to determine the presence of

extreme resistance genes (Ry) in potato germplasm. In one hand, the data reveals a clear harmony between the markers of *S. tuberosum ssp. andigena* and *S. stoloniferum* derived populations from across the PVY resistance variety (White Lady) and PVY susceptible genotype (W1100). When all two tested markers were present in the variety White Lady, while it was evident that those markers were not able to be detected in the genotype W1100, which are used as positive and negative controls respectively. This fact signifies that the knowledge of parental background of a clone is paramount for the utilization of markers that can detect when resistance gene(s) are genuinely present. However, degree of successfulness of molecular based evaluation for distinguishing characters of interest will be influenced by the genetic position between the marker in use and the gene of interest. This could also be coupled with their degree of genetic distance with the source of resistant genotypes like *Solanum tuberosum ssp. andigena* and *S. stoloniferum* and/or level of compatibility during intercrossing, besides the specificity of the marker.

In spite of the presence of generally agreed dominant fact which states "the closest the genetic marker to the gene of interest has the better detection efficiency" but in this particular study the degree of the genetic distance between the test material and the source of resistance genotypes and most probable their compatibility efficiency during intercrossing seemed to be the influential cause of the marker band strength. This is could be supported by the outcome of the RYSC3 marker, all tested genotypes could be screened as the carrier of the gene, which is closely linked (6.8cM) to the $Ry_{adg}$ gene, whereas only three genotypes are selected in the case of ST1 marker though it is closer to the $Ry_{sto}$ gene (1.3cM).

We also publicized those three genotypes of the institution (Cmm16, 96. 376 and FH79. 029. 02) carried $Ry_{adg}$ and$Ry_{sto}$ PVY extreme resistance genes, would be significant to incorporate them in the potato breeding schemes. On top of that, pyramiding with other PVY extreme resistance genes will enhance durability, on the other way; based on our experiment further evaluation of breeding lines would maximize effectivity of potato breeding in the Potato Research Centre.

## ACKNOWLEDGEMENTS

This work was supported by the Hungarian Government and FAO jointly.The author wishes to express his heartfelt to the Government of Hungary and the FAO for their generous fund provision to execute the project; and great thanks to the Community of University of Pannonia, Georgikon Faculty for hosting.

## REFERENCES

[1] http://faostat.fao.org/ 28 September, 2015/

[2] **Bendahmane, A., Kanyuka, K., & Baulcombe, D. C. 1999**. The Rx gene from potato controls separate Virus resistance and cell death response. *Pl cell, 11*: 781-791.

[3] **Boris , S. D., Monica, M. R., Claudia, B. P., Ivette, A. B., Julio, K. B., & Jose , S. R. 2008**. Evaluation of a SCAR RYSC3 marker of the $Ry_{adg}$ gene to select resistance genotypes to potato virus Y (PVY) in the INIA Potato breeding program. *FIA Biot., 1*: 1-15.

[4] **Brunt, A. A. 2001**. *The main Viruses infecting potato crops.* UK: Horticultural Research International, Warwicks CV 359EF.

[5] **Brunt, A. A., Grabtree, K., Dallwitz, M. J., Waston, L., & Zurcher, E. J. 1996**. *Plant viruses online description and lists from ViDE Data base.* Retrieved september 12, 2014, from http://image.fs.uidaho.edu/videl/.

[6] **Dalla Rizza, M., Francisco, L., Vilaro, Diego , G., Torres, & Diego, M. 2006**. Detection of PVY resistance genes in potato germplasms from the uruguayan breeding program. *Amer. J. Potato Res., 83*: 297-304.

[7] **De Bokx, J. A., & Hittinga, H. 1981**. Potato virus Y CIM/AAB Descri, P1 Viruses, No 242.

[8] **Dougherty, W. G., & Carrigton, J. C. 1988**. Expression and function of PotyViral gene products. *AnnRev.Phytopathol, 26*: 123-143.

[9] **FAO. 2004a**. The state of food insecurity in the world. Rome : FAO.

[10] **FAO. 2004b**. Human Energy Requirements, Reports of a joint FAO/WHO/UNU expret consaltation FAO Food and nutrition technical report series *No1*. Rome : FAO.

[11] **FAO. 2013**. Food supply. http://faostat3.fao.org/home/index.html. Date accessed: February. 17th, 2015.

[12] **FAO: Food and Agricultural organization. 2008**. http://www.fao.org/potato 2008/en/world/africa.html. Rome, Italy: FAO.

[13] **FAOSTAT. 2008**. http://wwww.potato.org/en/world/index.html. Rome, Itay: Food and Agricultural organization Stastical data base.

[14] **FAOSTAT. 2011**. Crops production . Rome, Italy: http://faostat.fao.org.

[15] **Fuglie, K. O. 2007**. Priorities for potato research in developing countries: Results of Survey. *Am.J Potato Res, 84*:353-365.

[16] **Gebhardt, C., Bellin, D., Heselewski, H., Lehmann, W., Schwarzfischer, J., & Valkonen. 2006**. Marker-assisdet combination of major genes for pathogen resistance in potato. *Theor. Appl. Genet., 112*: 1458-1464.

[17] **Hámáláinen, J. H., Watanable, K. W., Valkonen, J. P., Arihara, A., Plaisted, R. L., Pehu, E., et al. 1997**. Mapping and marker -assisted selection for a gene for extreme resistance to potato Virus Y. *Theor Appl Genet, 94,*:192-197.

[18] **Harison, B. D. 1984**. CMI/AAB Description of plant viruses Potato leaf roll virus 291(No. 36 revised). www.dpwed.net/dpv/showdpv.php?dpvno291.

[19] **Harverkot, K., P.C Struik, R., & Jacobesen, F. V. 2009**. Applied biotechnology to combat late blight in potato caused *Phytophthora infestans*. *Potato Res, 52*:294-264.

[20] **Jonathan, J., Whitworth, Richard, G., Novy, Darren, G., Hall, et al. 2009**. Charactrization of broad spectrum potato virus Y resistance in *Solanum tuberosum ssp*. andigena-derived population and select breeding clones using molecular markers, grafting and field inoculation. *Am J .Pot .Res., 86*: 286-296.

[21] **Kasai, K. Y., Morikawa, V. A., Sorri, J. P., Valkonen, Gebnardt, C., & Watanabe, K. N. 2000**. Development of SCAR markers to the resistance gene Ryadg based on a common feature of plant disease genes. *Genome , 42*: 1-8.

[22] **Kerlan, C., Tribodel, M., Glais, L., & Guillet, M. 1999**. Infections in vivo and in vitro transcripts from a full-lenght of cDNA clone of PVYN605, a swiss necrotic isolate of potato Virus Y. *J.Gen. Virol., 78*: 3141-3143.

[23] **Macrczewski, W., Strzelczyk-Zyta, D., Hennig , J., Witek, K., & Gebhardt, C. 2006**. Potato chromosomes IX and XI carry genes for resistance to potato virus M. *Theor. Appl .Genet., 112*: 1232-1238.

[24] **Michovilovich, E. J., Salazar, L. F., Saguma, F., & Bonierbale, M. W. 1998**. Survey the durability of extreme resistance to PVY derived from *Solanum tuberosum ssp*. andigena. Lima Peru: Páginas 123-128 CIP program report 1997-1998.

[25] **Missiou, A., Kalantidis, A., Tzorakaki, K., Tabler, S., & Tsagris, M. M. 2004**. Generation of transgenic potato plants highly resistance to potato virus Y (PVY) through RNA silencing. *Mol. Breeding , 14*: 185-197.

[26] **Ottoman, R. J., Hane, D. C., Brown, C. R., Yilma, S., James, S. R., Mosely , R. A., et al. 2009**. Validation and implementation of marker-assisted selection (MAS) for PVY resistance (Ry gene)in tetraploid potato breeding. *Am.J of Potato Res., 86*: 304-314.

[27] **Robaglia, C., Durand, M., Tronchet, M., Boudazin, G., Astier-Manifacier, S., & Casse-Delbart, F. 1989**. Nucleotide Sequence of Potato Virus Y (N strain) genomic RNA. *Journal of General Virology, 70*: 935-947.

[28] **Ross, H. 1986**. Potato breeding. Problems and prespectives. *Adv. Plant Breed(suppl.)*, 13.

[29] **Sigvald, R. 1984**. The relative efficiency of some aphid species vector of potato Viurs Y. *Potato Res., 27*: 285-290.

[30] **Smith, K. M. 1931**. Composite of certain potato viruses of the mosaic gruop. *Nature*, 127-702.

[31] **Tankesly, S. D., AH, P., & Bonierbale, a. M. 1989**. RFLP mapping in the plant breeding. *New tool for an old Scinence Bio Technology, 7*: 257-264.

[32] **Varvarre, D. A., Goyer, A., & Shakya, R. 2009**. Nurtitional Value of Potatoes: Phytonutrient and mineral Content. *Academic Press New York Unite States of America*(Singh,J. and L Kaur (eds)), 395-424.

[33] **Walbot, V., & Warren, C. 1988**. Regulation of Mu-element copy number in maize lines with an active or in active Mutator transposable element systems. *Mol. Gen Genet., 211*: 27-34.

## AUTHORS

**First Author** – MollaGeremeTaye, Department of Plant Biotechnology, Tigray Agricultural Research Institute, Mekelle, Ethiopi, Corresponding author email address: mogereme1982@gmail.com