Development of Early Blight and Late Blight Resistant Tomatoes

Report of a research supported by:

The Pennsylvania Vegetable Marketing and Research Program

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Long-range goal of the Penn State tomato genetics and breeding program:

Develop tomato cultivars with strong disease resistance (in particular early blight and late blight), improved fruit quality, and adaptation to PA conditions (e.g. high yield).

Specific objectives of 2010 project:

1. Fine mapping and characterization of new late blight (LB) resistance genes.

2. Transfer of new LB resistance genes to fresh-market cherry and grape tomatoes.

3. Transfer of new LB resistance genes to large size fresh-market tomatoes.

4. Transfer of new LB resistance genes to processing tomatoes.

5. Transfer of Ph-2 and to Ph-3 to large size fresh-market tomatoes.

6. Transfer of Ph-3 to processing tomatoes.

7. Continue breeding for early blight resistance.

Funds Received in 2010 From PVMRP: $4,000

2010 Research Progress:

(Please note that the research described below is a summary of research conducted during 2010 (and some prior to 2010) in the tomato genetics and breeding program at Penn State with funds received from different sources, including the $4,000 from the PVMRP. While funds from PVMRP have been very helpful, not all of the research described below was the result of such funds.)

Summary: We continued our research toward understanding the genetic bases of early blight (EB) and late blight (LB) resistance and developing tomatoes with disease resistance, high fruit quality, and adaptation to PA conditions. The work included laboratory research, (e.g. identifying polymorphic genetic markers, genetic mapping of new markers, determining genomic location of genes or QTLs), greenhouse research (e.g. growing plants for cross hybridization, disease resistance evaluation, preparing seedlings for field trials), field research (e.g. evaluating plants for numerous horticultural characteristics and making selections) and office work (e.g. data analysis and preparation of manuscript). During the summer 2010, for example, we grew approximately 8 acres of tomato breeding materials at the Russell E. Larson Agricultural Research Center at Rock Springs, PA, which were evaluated for disease resistance and many other characteristics. The field research resulted in the selection of hundreds of entries for further evaluation or cross-hybridization (including development of experimental hybrids and new breeding populations). A brief description of some of the experiments conducted in 2010 (and some prior to 2010) is as follow.

Identification, mapping and fine mapping of new late blight resistance genes

This has been a multi-year project and background information on this project was provided in our previous research reports. Briefly, we had identified a few accessions of the wild species of tomato *S. pimpinellifoium* L. with strong resistance to multiple isolates of *Phytophthora infestans*, the causal agent of tomato late blight. We had chosen one accession (PSLP153) for genetic mapping and identification of gene(s) conferring resistance. A project was initiated to identify and map gene(s) conferring LB resistance in PSLP153. One major part of this mapping project was identifying molecular markers polymorophic between PSLP153 and the cultivated parent (NCEBR-2) of the mapping population. This was proved to be very challenging due to close genetic relationship between PSLP153 and NCEBR-2. Thus, major efforts were devoted to this process and we identified a good number of polymorphic markers. The original mapping project, conducted in an F_2 population, indicated the presence of possibly 2 resistance genes, one on chromosome 1, tentatively named *Ph-5*, and one on chromosome 10, yet unnamed. The goal of this project was to fine map these two resistance genomic locations by developing near-isogenic lines (NILs).

Several generations of marker-assisted selection (MAS) and breeding as well as phenotypic selection resulted in the identification of one F_4BC_2 plant, highly similar to the recurrent parent, NCEBR-2 (i.e., with minimal background contribution from PSLP153), LB resistant, and heterozygote at the two target (resistance) regions on chromosomes 1 and 10 (as determined by 3 molecular markers). Additional generations of marker-assisted selection along with phenotypic selection (i.e., via disease screening) have lead to the development of advanced backcross populations (i.e., $F_4BC_3S_1$ and F_4BC_4). The populations are in fact near-isogenic lines, and are being used to fine map the genes on chromosomes 1 and 10. The identified NILs will be useful for transferring the resistance to different genetic backgrounds without having to use the original resistant wild parent (PSLP153). The NILs will also be useful for determining the individual effects of each new gene identified as well as their effects in homozygous or heterozygous conditions. Furthermore, the NILs will facilitate further fine mapping and possibly cloning the new resistance genes. In addition to determining the exact locations of the new resistance genes and developing NILs, a major goal of this project has been transferring of these resistance genes to our tomato breeding lines via marker-assisted selection (MAS), as described below.

Breeding fresh-market cherry and grape tomatoes for late blight resistance using the new late blight resistance genes

A major goal of this project has been to develop cherry and grape tomatoes with late blight (LB) resistance introduced from the LB-resistant *S. pimpinellifolium* accession PSLP125. Briefly, during greenhouse experiments in early 2006, F_2 individuals of the cross between PSLP125 and tomato breeding line NCEBR-2 were evaluated for LB resistance. F_2 individuals exhibiting strong resistance to LB were selected and self-pollinated to produce F_3 progeny. The F_3 progeny families were grown under field conditions in 2006 and screened for various horticultural characteristics. At the end of the 2006 field season, a number of F_3 plants were selected carrying desirable horticultural characteristics. Selected individuals were self-pollinated to produce F_4 seed. During early 2007, F_4 families were evaluated for LB resistance in the greenhouse. Phenotypically resistant individuals from the selected families were also marker genotyped to assure presence of the resistant segment on chromosome 1 in homozygous conditions (note that at that point we knew only of this resistant segment and not about the segment on chromosome 10). During the summer 2007, the F_4 families were grown under field conditions and evaluated for horticultural characteristics. Comprehensive data were collected as to the various characteristics of these selections. The goal was to identify individuals with good prospect for development of inbred lines of cherry and grape tomatoes with LB resistance. At the end of the season, 3 cherry and 2 grape F_4 plants (each from one F_4 family) were selected, self-seed of which (F_5 generation) were collected. These F_5 families were all expected to be homozygous for LB resistance genes, however, F_5 seed was collected from one plant per F_4 family. In spring 2008, the five F_5 families were grown in greenhouse and evaluated for LB resistance. As expected, all families were completely resistant. Six plants per family were selected (those with other apparent desirable characteristics) and seeds were bulk-harvested per family. During the summer 2008, the five F_6 families (3 cherry and 2 grape) were grown under field conditions. These plants were evaluated for various characteristics, including plant type and size, fruit size and shape, fruit color and taste and stem joint (*jointed* vs. *jointless*). Following evaluations, six inbred lines (3 cherry and 3 grape) were selected and named as follow: PSCHLB-1, PSCHLB-2, PSCHLB-3, PSGRLB-1, PSGRLB-2 and PSGRLB-3 (this one was selected from segregation in PSCHLB-1). The six selected lines were self-pollinated and produced progeny for the next cycle of field evaluation. During winter/spring 2009 several new experimental hybrids were produced from crosses between the three LB resistant grape lines (i.e. PSGRLB-1, PSGRLB-2 and PSGRLB-3) and 11 Penn State grape lines with high lycopene and other desirable characteristics (total of 33 experimental hybrids). However, as the hybrid seeds did not become available before field season, they were not included in the 2009 field trial (they were included in the 2010 field trial; see below). During 2009 field season, however, the six inbred lines were again grown under field conditions and evaluated for various horticultural characteristics. From these evaluations, two lines, namely PSCHLB-2 and PSGRLB-3 that were overall better than others, were selected for developing experimental hybrids. During winter/spring 2010, these two lines were grown under greenhouse conditions and crossed with 5 Penn State cherry and 9 Penn State grape tomatoes (for a total of 28 experimental hybrids). During the 2010 field season, in addition to growing the six LB-resistant cherry and grape lines, we grew the 33 and 28 experimental hybrids that were developed in spring 2009 and 2010, respectively. During the field 2010, these inbred lines and hybrid were evaluated for various horticultural characteristics and decisions were made as to their further improvement. The future goal of this project is to develop commercial hybrids of cherry and grape tomatoes with LB resistance. At the time of writing of this report, we are making

crosses between two Penn State LB-resistant grape tomatoes and 7 complementary lines to develop 14 experimental hybrids of grape tomatoes for field evaluation and potential release. If any of these hybrids looks competitive, we will release for commercial production. However, the six inbred lines of cherry and grape tomatoes with LB resistance we have developed are also ready for release to seed companies for commercial evaluation.

Breeding processing and large-size fresh-market tomatoes for late blight resistance using the new late blight resistance genes

As mentioned in the above section on mapping late blight resistance genes, our recent research indicated that there might be two late blight (LB) resistance genes in the wild *S. pimpinellifolium* accession PSLP153, one on chromosome 1 (tentatively named *Ph-5*) and one on chromosome 10 (unnamed yet). Our goal has been to transfer these resistance genes to our fresh market and processing tomatoes. As described in the previous section, this has been a multiyear project, starting a few years ago. First we developed F_2, F_3 and F_4 populations from crosses between LB-resistant *S. pimpinellifolium* accession PSLP153 and tomato breeding line NCEBR-2. Several generations of backcrossing, self-breeding and selection (both phenotypic selection and marker-assisted selection) resulted in the development advanced populations grown in the field in 2009. From field 2009, 15 plants (3 processing types and 12 fresh-market types) were selected and self-pollinated and BC_2S_2 seeds were collected. To continue this project toward development of processing and large size fresh-market tomatoes with LB resistance from PSPL153, the following two sets of activities were initiated.

1. In the spring of 2010, the three processing and 9 (of the 12) fresh-market BC_2S_2 families were grown in the greenhouse and screened for LB resistance. Plants with apparent LB resistance were saved and subsequently transplanted into the field (specifically, total of 9 plants from the three processing families and 27 plants from a total of 8 fresh-market families). The following selections were made during the field 2010 in processing and fresh market type tomatoes:

Processing families: Following disease screening in the greenhouse, three (3) resistant processing plants from each family were planted in the field. Evaluation conducted in the field for horticultural (keeping in mind the % defoliation and marker genotype), resulted in the selection of a total of 4 plants for further breeding work. The selected plants were self-pollinated and collected BC2S3 seeds. At the time of writing this report, these materials are being screened for LB resistance in the greenhouse. We will identify the most resistant plants, which will be genotyped for LB-linked markers on chromosomes 1 and 10. LB homozygous resistant plants will be self-pollinated and hybridized with superior processing lines developed at Penn State for further improvement in the horticultural characteristics of these lines, as will be described in our 2011 research proposal.

Fresh-market families: Following disease screening in the greenhouse, 3 to 6 resistant plants from each of the 9 families were planted in the field. Evaluation conducted in the field for horticultural (keeping in mind the % defoliation and marker genotype) resulted in the selection of a total of 7 plants for further breeding work. The selected plants were self-pollinated and collected BC2S3 seeds. At the time of writing this report, these materials are being screened for LB resistance in the greenhouse. We will identify the most resistant plants, which will be genotyped for LB-linked markers on chromosomes 1 and 10. LB homozygous resistant plants will be self-pollinated and

hybridized with superior fresh-market lines developed at Penn State for further improvement in the horticultural characteristics of these lines, as will be described in our 2011 research proposal.

2. In the spring of 2010, one processing and one fresh market family, which were homozygous for the LB-resistance-linked markers on both chromosome 1 and 10, were selected for further backcrossing. The processing family was hybridized with 6 Penn State processing lines, and the fresh-market family was hybridized with 5 Penn State fresh-market lines. The resultant 11 BC3 progeny families (all of which supposedly heterozygous for the two resistance segments on chromosomes 1 and 10) were planted in the field. The following selections were made during the field 2010 in processing and fresh market type tomatoes:

Processing families: The six BC_3 processing families were evaluated for horticultural characteristics under field conditions, and a total of 3 best plants were selected and self-pollinated to produce BC_3S_1 seed. At the time of writing of this report, the 3 select BC_3S_1 processing families are grown under greenhouse conditions to be evaluated for LB resistance. At this point these plants should segregate for resistance. Following the identification of the most resistant plants, they will be marker genotyped to identify homozygous resistant for both segments on chromosomes 1 and 10. Following identification of homozygous resistant plants, they will be used in two ways. First, they will be self pollinated and advanced to the next generation. The progeny generation (BC_3S_2) will be transplanted in field 2011 for further evaluation and breeding, as will be described in the 2011 research proposal. Second, the select homozygous LB resistant plants will be hybridized with superior PSU processing lines for further improvement, as will be described in the 2011 research proposal.

Fresh-market families: Five BC_3 fresh-market families were evaluated for horticultural characteristics under field conditions in 2010, and a total of 8 best plants were selected and self-pollinated to produce BC_3S_1 seed. At the time of writing of this report, the 8 select BC_3S_1 fresh-market families are grown under greenhouse conditions to be evaluated for LB resistance. At this point these plants should segregate for resistance. Following the identification of the most resistant plants, they will be marker genotyped to identify homozygous resistant for both segments on chromosomes 1 and 10. Following identification of homozygous resistant plants, they will be used in two ways. First, they will be self pollinated and advanced to the next generation. The progeny generation (BC_3S_2) will be transplanted in field 2011 for further evaluation and breeding, as will be described in the 2011 research proposal. Second, the select homozygous LB resistant plants will be hybridized with superior PSU processing lines for further improvement, as will be described in the 2011 research proposal.

Breeding processing and large-size fresh-market tomatoes for late blight resistance using previously-known late blight resistance genes, Ph-2 and Ph-3

A goal of this project has been to incorporate the previously-identified LB resistance genes, *Ph-2* and *Ph-3*, into our fresh-market and processing tomatoes. Background information on this multi-year breeding project was given in our previous research reports. In below, the progress to develop fresh-market and processing tomatoes with *Ph-2* and *Ph-3* resistance is described:

Development of FM tomatoes with Ph-2 and Ph-3 resistance: As indicated in previous research reports, a tomato breeding line was used as the source of *Ph-2* and *Ph-3* resistance. Originally, several Penn State tomatoes were hybridized with this line and produced F3 (from crosses in 2007) and F4 (from crosses in 2006) families. Selected F4 individuals (i.e., LB resistant and homozygous for *Ph-3*) were hybridized with two other tomato genotypes and produced F4BC1 families. A total of 37 F4BC1 families were developed, which were grown in the greenhouse, self-pollinated and produced BC1S1 seed. Similarly, the F3 families were both self-pollinated to produce "new" F4 families and crossed with a line to produce F3BC1 families. During the spring 2009, four sets of families were grown in the greenhouse, evaluated for LB resistance and genotyped for *Ph-3* resistance. Only plants which were either homozygous or heterozygous for *Ph-3* were planted in the 2009 field, where they were evaluated for various horticultural characteristics and a total of 31 plants were selected from the four families. These plants were self-pollinated and advanced to the next generation. To continue this project toward development of processing and large size fresh-market tomatoes with LB resistance conferred by *Ph-2* and *Ph-3* genes, the following two sets of activities were initiated in 2010.

1. In the spring of 2010, further inspection of the 31 selected families resulted in the selection of 7 families for further studies. Twelve plants of each family were grown in the greenhouse and screened for LB resistance. Resistant plants from each family (ranging from 0 to 12 plants per family) were planted in the field 2010. Evaluation conducted in the field for horticultural (keeping in mind the % defoliation and marker genotype) resulted in the selection of a total of 6 plants from three families (which exhibited the most resistance), which were self-pollinated to produce the next generation. The goal of this project is to develop fresh-market lines homozygous at both *Ph-3* and *Ph-2* and with other desirable horticultural characteristics. At the time of preparing this report, the 6 selected progeny families are grown in the greenhouse to be evaluated for LB resistance. We will identify the most resistant plants, which will be genotyped for LB-linked markers for *Ph-3* and possibly *Ph-2*. LB homozygous resistant plants will be identified for further breeding activities, as will be described in the 2011 research proposal.

2. In the spring of 2010, 4 of the 7 selected fresh-market families, which all were homozygous homo for *Ph-3* and unknown for *Ph-2*, were selected for further backcrossing. These fresh-market lines were hybridized with 5 Penn State fresh-market lines, resulting in 20 progeny families (all supposedly heterozygous for *Ph-3*), which were planted in field 2010. Evaluation were conducted in the field for horticultural characteristics, which resulted in the selection of a total of 9 plants from 8 families. At the time of preparing this report, the 9 selected LB resistant families are grown in a greenhouse to be evaluated for LB resistance. We will identify the most resistant plants, which will be genotyped for LB-linked markers for *Ph-3* and possibly *Ph-2*. LB homozygous resistant plants will be identified for further breeding activities, as will be described in the 2011 research proposal.

Development of processing tomatoes with Ph-3 resistance: A goal of this project was to transfer a previously-identified LB resistance gene, *Ph-3*, into Penn State processing breeding lines. Thus, in 2006 a processing tomato line, supposedly containing *Ph-3* in homozygous condition, was hybridized with several of our breeding selections and the progeny families were grown under field conditions during summer 2006. Unfortunately, most families did not look desirable, as they were more like fresh-market tomatoes with small round fruits. No selection was made from 6 families and only 5 individuals were selected from 3 families. The 5 families were grown in the greenhouse

during late 2006 and evaluated for LB resistance in January 2007. Most plants within families exhibited LB susceptibility. Evaluations resulted in the selection of 10 plants with apparent resistance. These 10 plants were grown to maturity and marker genotyped for *Ph-3* markers. The 10 plants were also hybridized with a processing tomato genotype. A total of 8 BC_1 progeny families were developed (did not get BC_1 progeny from two of the crosses). The 8 BC_1 families were grown under field conditions in 2007 and were evaluated for horticultural characteristics. We selected a total of 9 plants from 4 different families. Plants were selected only from families in which the parental PSU parents were homozygous for *Ph-3* markers. Thus, it could be assumed that these BC1 families were heterozygous for *Ph-3* marker. The BC1S1 seeds were collected from the selected BC1 families in the field. In spring 2008, the 9 BC1S1 families (collected from field 2007) were evaluated for LB resistance in the greenhouse. Of these, 4 families were dropped as they were susceptible. From each of the remaining 5 BC1S1 families, 2-5 resistant plants were grown to maturity. The plants were genotyped for *Ph-3* markers. From these 5 families, a total of 11 BC1S1 plants were found to be homozygous for *Ph-3*. Subsequently, each of these 11 plants were hybridized with two desirable processing genotypes. BC2 seeds were obtained from 11 crosses with one and 10 crosses with the other processing parent. So we developed a total of 21 new BC2 families. In addition to these crosses, pollens were pooled from individuals coming from each of the 5 BC1S1 families and hybridized with each of the two desirable processing genotypes, and thus developed another 10 new BC2 families. Thus, altogether we developed a total of 31 BC2 families, all of which were expected to be heterozygous for *Ph-3*. The BC2 families were grown under greenhouse conditions and evaluated for LB resistance. Only highly resistant families were maintained, and self-pollinated to produce BC2S1 families. A few families were lost due to unforeseen situations and a few families did not show sufficient resistance. Thus, there remained 20 BC2S1 families for further research activities. In winter 2009/2010 these 20 families were grown under greenhouse conditions (12 plants of each family, for a total of 240 plants). They were evaluated for LB resistance, and the resistant individuals were genotyped for *Ph-3*. From among the different families, we identified 12 plants with high resistance to LB and homozygous at *Ph-3*.

The 12 BC2S1 selected plants had different backgrounds, that is, whether the second BC was with a Heinz (HZ) or a TSH hybrid genotype. Thus, they were divided into two groups, BC2S1-HZ and BC2S1-TSH. These two groups of plants were grown to maturity and pooled pollens were collected from each group. The pooled pollen was used to hybridize 6 select Penn State processing tomato breeding lines and thus produced 12 new BC3 families. All these families were expected to be heterozygous for *Ph-3* and otherwise vary in other traits. Twelve plants of each family (for a total of 144 plants) were plant in the field 2010. These families were evaluated for horticultural traits, and a total of 12 best plants were selected. At the time of preparing this report, the 12 selected LB resistant families are grown in a greenhouse to be evaluated for LB resistance. We will identify the most resistant plants, which will be genotyped for LB-linked markers for *Ph-3*. LB homozygous resistant plants will be identified for further breeding activities, as will be described in the 2011 research proposal.

Development of processing and fresh-market tomato breeding lines with disease resistance and high fruit quality via traditional protocols of plant genetics and breeding and contemporary techniques of marker-assisted selection

This has been an ongoing research for several years. Each year many large-scale greenhouse and field experiments are conducted with the aim of transferring resistance genes and other desirable horticultural characteristics to our processing and fresh-market tomato breeding lines. We have been advancing hundreds of lines and developing hundreds of new breeding populations each year and evaluating them under field conditions. For example, for the past few years, during each field season we grew and evaluated an average of 8-10 acres of experimental tomatoes at Rock Springs. Each season we grew breeding populations, families and lines, which were at different stages of breeding advancement. They were also of both fresh-market and processing types. The fresh-market types included cherry, grape, plum-shape and large-size round tomatoes. Each season, plants were evaluated for numerous characteristics, including disease resistance, fruit quality, maturity, yield, plant type, etc. and usually several hundred selections were made, including processing selections, fresh market selections (such as large and medium round tomatoes, plum tomatoes, cherry tomatoes, and grape tomatoes). Normally the selected individuals are self-pollinated to advance them to the next generation. Many of these selections normally grown during the winter and spring time in the greenhouse for further crosses and development of new populations, which would be grown in the field the next year. As an example, the activities during the 2010 field season are summarized below.

Crosses were made during the winter/spring 2010 and altogether we developed a total of 93 new populations/hybrids. During the growing season in 2010, the following materials were planted in the field:

A. From 2009 Field

262 plots of processing lines
190 plots of large size fresh-market lines
51 plots of plum fresh-market lines
25 plots of cherry fresh-market lines
29 plots of grape fresh-market lines

B. From 2009 Crosses (Experimental Hybrids)

84 plots of plum fresh market hybrids
60 plots of cherry fresh market hybrids
73 plots of grape fresh market hybrids

C. From 2010 Crosses (Transferring LB Resistance)

9 plots of large size fresh-market tomatoes segregating for *Ph-5* LB resistance
3 plots of processing tomatoes segregating for *Ph-5* LB resistance

7 plots of large size fresh-market tomatoes segregating for *Ph-3* LB resistance

3 LB-resistant cherry lines (*Ph-5* resistance)
3 LB-resistant grape lines (*Ph-5* resistance)

6 plots of new processing hybrids developed from a *Ph-5*-resistant line and 6 PSU lines
5 plots of new fresh-market hybrids developed from a *Ph-5*-resistant line and 5 PSU lines

12 plots of new processing hybrids developed from two *Ph-3*-resistant pools and 6 PSU lines
20 plots of new fresh-market hybrids developed from four *Ph-3*-resistant lines and 5 PSU lines

D. Commercial Breeding Lines and Hybrids
41 plots of commercial fresh market lines/hybrids
18 plots of commercial fresh market lines/hybrids

During the field 2010, all of these materials were evaluated for various horticultural characteristics. We have made significant selections from within/among different populations/families/lines, which have been self-pollinated for generation advancement. These materials will be used for different purposes, including some release, further hybridization and/or further evaluation in the future. In summary, this multi-year project has resulted in the development of advanced processing and fresh-market tomato breeding lines with numerous desirable horticultural characteristics, including disease resistance (mainly early blight and late blight), fruit quality (in particular high fruit lycopene content), high yield, early maturity and otherwise adapted to PA conditions. Thus far, several fresh-market breeding lines (of plum, cherry and grape types) have been released to several seed companies, which are currently evaluating their commercial value. We expect that we will be able to release more breeding lines, including processing and large-size fresh market tomatoes, with desirable horticultural characteristics suitable for production in PA within the next few years, assuming that research funding will be available.

Conclusions

We have continued our research toward genetic characterization of EB and LB resistance in tomato and development of new tomato inbred lines and experimental hybrids with improved disease resistance and other desirable horticultural characteristics, including high fruit lycopene content. Specifically, we have 1) identified resistant wild accessions of tomato with resistance to EB and LB, 2) developed several interspecific populations of tomato segregating for disease resistance, 3) characterized genetic controls of EB and LB resistance including the identification of QTLs controlling EB resistance, 4) developed new tomato germplasm, including inbred lines and experimental hybrids, with improved disease resistance and other desirable horticultural characteristics, 5) have released several fresh market and a few processing tomatoes with improved EB resistance and high fruit lycopene content, and 6) will releasing more lines and hybrids within the next few years with resistance to LB as well as other desirable horticultural characteristics.

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Budget:

The actual expenses for the projects described above were much higher than what was provided by the Pennsylvania Vegetable Marketing and Research Program. However, financial support from growers is highly appreciated and it is valuable and important to the continuation of this long-term research. Below the *approximate* expenditures in this project:

Wages (including fringe benefits)	$3,500
Greenhouse and field rent	$500
TOTAL	$4,000

Duration of Project: 8 months (April 1, 2010 to November 30, 2010)