AGRICULTURE AND BIOLOGY JOURNAL OF NORTH AMERICA
ISSN Print: 2151-7517, ISSN Online: 2151-7525, doi:10.5251/abjna.2011.2.12.1409.1415

Identification of suitable parents and temperatures for breeding Potato virus Y (PVY) and Potato virus X (PVX) resistant potatoes

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ABSTRACT

Mixed infection of Potato virus Y (PVY) and Potato virus X (PVX) together with other potato viruses have the potential of causing yield losses of up to 80 % in the major varieties grown in Kenya. In search for suitable resistant parents and a favourable temperature range for cross breeding, seven virus resistant potato genotypes from International Potato Centre (CIP), Lima Peru were test crossed (progeny tested) with one local PVY and PVX susceptible cultivar (Tigoni) under low (11-18°C), medium (18-27°C) and high temperatures (28-34°C) at the Kenya Agricultural Research Institute (KARI), Tigoni. Low temperature range of (11-18°C) with the highest percentage (43%) of successful crosses was identified as the most favourable for cross breeding. Among the seven CIP clones tested, CIP395196.4 gave the highest percentage (98 %) of resistant progenies and was found to have multiplex Ry Ry Ry ry / Ry Ry Ry Ry genes for PVY and PVX resistance. This type of parent was the most suitable for cross breeding because it produced significantly (P=0.05) 100% resistant progenies when crossed with a susceptible cultivar hence eliminating the need (cost and labour) for preliminary seedling screening.

Keywords: Potato, PVY, PVX, parents and temperature

INTRODUCTION

Potato crop makes a significant contribution to food security in Kenya as the second most widely grown food crop after maize (MoA/GTZ-PSDA, 2009). Although it is an important crop, the national average of 4.4 T/ha is low compared to the world average of 17 T/ha in developed and 13 T/ha in developing countries (FAO, 2009). Without the constraints in potato production, it is possible to realize 40 T/ha under research station conditions in the country (Lung'aho *et al.,* 2008). Among the major constrains of potato production, potato viruses are of major concern because of the role they play in reducing crop quality and productivity (Boiteau *et al.,* 1988; Kabira *et al.,* 2006a).

In Kenya, potato viruses responsible for major yield reductions include Potato virus Y (PVY), Potato virus (PVX) and Potato leaf roll virus (PLRV), which occur in combination with other mild viruses cause more severe yield losses and the major varieties grown do not have effective resistance against such viruses (Kabira *et al.,* 2006b). Disease severity increases once the crop is infected leading to successive yield and crop quality losses over the seasons (Lung'aho *et al.,* 2007). A past survey reported high prevalence of potato viruses in the major potato growing areas in Kenya where multiple infections of PVY and PVX in combination with PLRV and other mild viruses caused major yield losses (Machangi *et al.,* 2004).

Several disease control measures for controlling viruses on potato crop have been attempted though the use of resistant varieties is the only recommended sustainable long-term solution to the yield reductions resulting from infection by these viruses (Khurana, 2000; Seed Potato Sub-Sector in Kenya, 2009). Among the types of potato virus resistance, extreme resistance which has been found particularly for PVY and PVX and is effective against all strains of the respective viruses and is considered to be the most durable type of resistance (Bradshaw and Mackay, 1994). The genes for this type of resistance confer the plant with the ability to show no symptoms, or show only limited necrosis (e.g. pinpoint lesions, flecks, or localized stem necrosis), when inoculated with virus and only extremely low amounts of virus, if any, can be detected by sensitive techniques (Solomon-Blackburn and Barker, 2001). A vital step for breeding for this type of resistance is progeny testing for identification of suitable sources

of resistance to use as parents (Solomon-Blackburn and Mackay, 1993; Song *et al.,* 1991). This involves conducting controlled crossing to get true potato seeds (TPS) for screening.

Obtaining TPS from crosses however, depends on both genetic and environmental factors during the time of crossing (Sekara and Bieniasz, 2008). Temperature is a major environmental factor affecting the process of pollination and fruit development in potato (Sleper and Poehman, 2006; Roy, 2007). Extreme low or high temperatures damage flower organs, interfere with pollen formation and reduce pollen viability and hence affect seed and fruit development (Sukhvibul *et al.,* 2005; Strand, 2008). To avoid the effect of temperature during pollination, cross breeding is usually conducted in glasshouses where controlled favourable temperatures are maintained (Burton, 1989; Aquaah, 2007).

Such facilities for controlling temperatures may not be available in many developing countries (Robinson, 2010) and breeders may be forced to conduct crosses only when the ranges of environmental temperatures are favourable. This study was therefore conducted to determine the optimum range of temperature for successful berry formation during cross breeding and also to identify the best parent among the CIP PVY and PVX resistant clones that can be used for crossing with the local susceptible cultivars to develop PVY and PVX resistant varieties.

MATERIALS AND METHODS

The study was conducted at KARI-Tigoni during Long rains (LR) 2008 and Short rains (SR) 2008. Potato clones (*Table 1*) with varying levels of PVY and PVX resistance obtained from the germplasm collection at CIP Lima, Peru were used as male parents while variety Tigoni which is one of the most recently released highest yielding Kenyan variety but PVY and PVX susceptible was used as the female parent.

Tubers were planted in the glasshouse and once the plants had flowered and were ready for pollination, pollen was collected by tapping the anther of the male parent on a glass slide. Emasculation of the female parent was done by breaking the anthers backwards using forceps carefully not to touch the stigma when removing the anthers when the petals were just about to separate but was still enclosed (Accantino and Malagamba, 1982; Robinson, 2010). The collected pollen was dusted on the stigma of the female parent. Fifty (50) flowers in 4 replications were crossed at 3 different temperature ranges (low, 11-18°C; medium, 19-27°C and high, 28-34°C) in the glasshouse that were recorded 36 hours after conducting the crosses. Two hundred crosses of each of the pedigrees were conducted. Numbers of successful crosses under the different temperature ranges were recorded.

Table 1: Pedigree of potato clones/varieties used

Code	Clone/variety	Pedigree of parents
1	394903.3	720118.1 x BWH87.183
2	395196.4	([C83.621 x KATAHDIN] x BULK 1-RKN
3	395438.1	BWH87.344R x TXY.11
4	396286.6	TXY.3 x 1-1039
5	396286.7	TXY.3 x 1-1039
6	394905.8	CRUZA -148 x C90.205
7	394904.17	720118.1 x (C90.205) 17
8	Tigoni	378493.915 x BULK PRECOZ

The potato fruits (berries) that developed were harvested 40 days after successful pollination. The berries were then kept at room temperature until they became soft for easy extraction of true potato seeds (Accantino and Malagamba, 1982). The extracted true potato seeds were treated with 1500 ppm of Gibberellic acid (GA_3) for 24 hours to break dormancy as recommended by Enrique and Fernandez (1991). Immediately after the GA_3 treatment, the TPS were washed and air dried and then sown in rows 1 cm deep and 2 cm between the rows in a substrate consisting of loam soil, manure, sand and ballast in the ratio 4:2:1:1 in the glasshouse. Between 1000 and 1500 seeds were sown from each set of cross.

Potato plant leaves confirmed using Enzyme Linked Immunosorbent Assay (ELISA) technique to be with combined infection of PVY and PVX and without infection by other viruses were used to prepare the sap extract. The leaves obtained from the infected plants were crushed between a sterile polythene sheets (2g of leaf tissue in 100 ml of distilled water). Caborundum powder (600 mesh) was sprinkled on tobacco plants, *Nicotiana occidentallis* and *Nicotiana glutinosa* and sterile swab was used to apply the infected sap on the 4-week-old tobacco leaves. The tobacco leaves were tested using ELISA to confirm PVY and PVX infection 3 weeks (21 days) after inoculation. The PVY and PVX infected leaves were

then used to prepare innoculum of 2 g of leaf tissue of equal amount of both *N. occidentallis* and *N. glutinosa* in 100 ml of distilled water (Dhiman *et al.,* 1994). The debris were separated from the sap by filtering using cheesecloth. The (sap) innoculum was then mixed with 0.5 % 600-mesh Carborundum powder (an abrasive powder) and then applied on the seedlings at 4 weeks after planting when the seedlings had developed around 3 to 4 leaves using painter's spray gun (Air compressor: Honda 5.5 GX 160, Japan**)** at a pressure of 25 psi at a distance of 2-3 cm away from the plant and spending 2-5 seconds per plant (Song *et al.,* 1991; Dhiman *et al.,* 1994). The plants were then immediately covered with a wet polythene sheet for 24 hours after inoculation (Dhiman *et al.,* 1994). The plants were monitored over the next 21 days for the development of mosaic and necrotic symptoms. At 21 days after inoculation, the number of healthy plants (plants without mosaic and necrotic symptoms) were recorded. The percentage of the healthy potato seedlings (without mosaic or necrotic symptoms) at 21 days after being subjected to dual PVY and PVX infection was calculated. The ratios of resistant to susceptible seedlings were subjected to Chi-square analysis to determine the suitable parents based on the deduced number of PVY and PVX resistance genes according to Caligari (1992).

RESULTS

Effect of temperature range on sucess of cross pollination: Variation in temperature range at the time of crossing affected percentage success of cross pollination, berry formation and fruit development. There was a significant (P=0.05) reduction in mean percentage of successful crosses from 43% to 8.3% to 0 % with an increase in temperature range from low to medium to high (*Table 2*).

Percentage berry formation ranged from 28 % to 85.5 % under low temperature range and from 0 % to 20 % under medium temperature range and none under high temperature range. The most favourable temperature range for cross breeding was a cool low temperature of between 11-18°C with up to 43% of successful crosses.

Thought it was possible to cross successfully at medium temperature, percentage of successful crosses was reduced from 43% to 8.3%. Very high temperatures did not favour successful cross pollination and berry development. In this experiment, it was not possible to obtain berries from crosses conducted above 28°C in all the sets of parental

genotypes used. Each varietal combination of parents crossed expressed their own prefered temperatutre range for sucessful cross pollination and berry developement. Some crosses gave higher percentage of success at low but not at higher temperature ranges.

Other clones gave higher percentage of successful crosses at medium temperature range but not at low temperature range. In crosses of (Tigoni X 394905.8), there was signifcant (P=0.05) higher percentage (85%) of succesful crosses at low temperature range compared to the rest of the crosses but not at higher temperature ranges. At medium temperature range, the crosses of (Tigoni X 394903.3) gave higher percentage (20%) of sucessful crosses than the most of the other crosses conducted but not at low temperature.

PVY and PVX resistance screening: The seven CIP clones in this experiment were a rich source of parental material for PVY and PVX resistance breeding with high percentage of resistant progenies when crossed with a local susceptible variety. Out of the 8400 seedlings screened, 81 % or 6804 seedlings were resistant to the two viruses. This high percentage of virus resistant seedlings are a useful germplasm collection for future evaluation of potential virus resistant varieties.

The most suitable parent for crossing with the local susceptible cultivars to give the highest percentage of potential virus resistant progenies among the 7 CIP clones tested was identified. The CIP clone 394196.4 in the cross (Tigoni x 394196.4) gave the highest percentage (98%) of healthy seedlings significantly (P=0.05) equivalent to (100 %) of seedlings compared to the rest of the crosses (*Figure 1*).

Other useful parental genotypes which can be used as subsitutes for cross breeding for virus resistance were also identified. These genotypes did not give 100% of resistant seedings but gave significantly (P=0.05) lower percentage (17%) of susceptible seedlings for screening. This was found in crosses between {Tigoni x 395438.1 (84%), Tigoni x 396286.6 (83%) and Tigoni x 396286.7 (83%)}.

Some clones were very difficult to cross, yielding lower percentage of succesful crosses (*Table 2*) and at the same time giving the lowest percentage of virus resistant progenies (*Figure 1*) when crossed

with a local susceptible cultivar. This included clones 394904.17 and 394903.3 which had the lowest (29% and 28% respectively) of succesful crosses and the lowest percentage of susceptible progenies (65 and 75 % respectively) when crossed with a local susceptible cultivar and hence were most insituable for breeding PVY and PVX resistance under the local conditions.

Parental combination without any of the PVY and PVX resistance genes were not at all useful for breeding PVY and PVX resistance. The seedlings obtained from such parents were not able to withstand disease pressure during the seedling screening and hence were all eliminated. This was found in the cross (Tigoni X Tigoni) with 2% of resistant seedlings which was significantly (P=0.05) equivalent to 0% of resistant seedlings after virus screening.

Estimating the number of PVY and PVX resistance genes in the parents: High number of resistance genes in parents corresponded with high percentage of resistant seedlings from a cross between a resistant parent and a susceptible cultivar. The best parent, CIP 395196.4 for breeding local varieties resistant to PVY and PVX was identified among the CIP clones tested. Seedlings screened from this cross (Tigoni X 395196.4) significantly (P = 0.05) fitted the 1:0 segregation ratio (*Table 3*) which when deduced according to Caligari (1992) had either triplex (Ry Ry Ry ry) or Quadruplex (Ry Ry Ry Ry) which is the highest possible ($^3/_4$ or $^4/_4$) number of genes in a resistant parent. Such parent will be the most useful for cross breeding with local varieties.

The next set of parents had ½ the number of the resistance genes and were not the best parents. Seedlings of the cross between (Tigoni X 396286.7), (Tigoni X 395438.1), (Tigoni X 394905.8), (Tigoni X 394903.3) and (Tigoni X 396286.6) which significantly (P = 0.05) fitted the 5:1 ratio were deduced to have been obtained from a duplex (Ry Ry ry ry) resistant parent which is deduced as a less suitable parent.

The parental cross among the CIP virus resistant clones with the lowest possible number of genes was (Tigoni X 394904.17) with with either none, ¼ or ½ of the single dominant genes (nulliplex-ry ry ry ry, simplex-Ry ry ry ry or duplex-Ry Ryry ry).

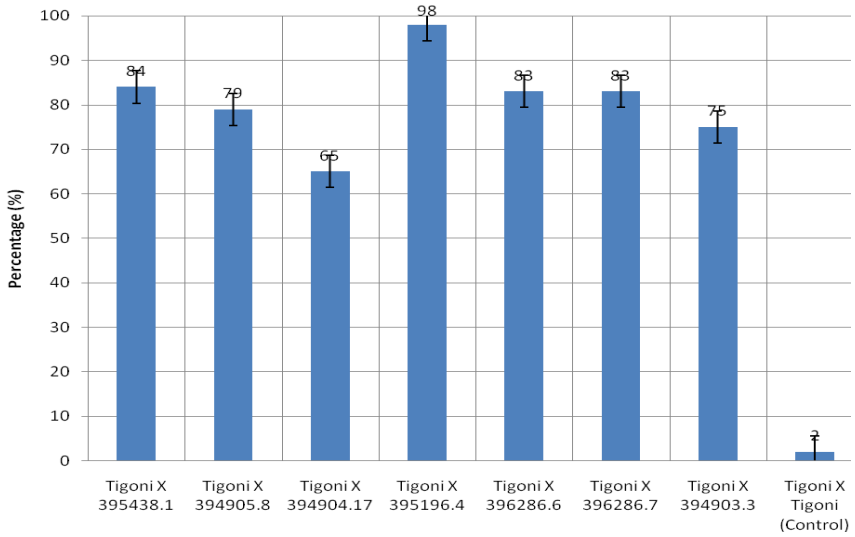

Fig 1: Percentage of healthy plants 21 days after inoculation with infective sap containing PVY and PVX

Table 2: Percentage of successful crosses under different temperature ranges 36 hours after crossing

Pedigree	Temperature range 36 hrs after crossing		
	Low (11-18 ˚C)	**Medium (19-27 ˚C)**	**High (28-34 ˚C)**
Tigoni X 395438.1	33	0	0
Tigoni X 394905.8	85.5	7.5	0
Tigoni X 394904.17	29	0	0
Tigoni X 395196.4	50	18	0
Tigoni X 396286.6	32.5	0	0
Tigoni X 396286.7	43	12.5	0
Tigoni X 394903.3	28	20	0
Tigoni X Tigoni	41	2	0
Mean	**43**	**8.3**	**0**
LSD (5 % level)	**6.3**	**6.3**	**6.3**

Table 3: Estimation of the number of resistance genes PVY and PVX resistance genes (compared to the expected χ^2 values of each segregation ratio)

Pedigree	χ^2 values at each segregation ratio			Genotype of resistant parent	No. of genes out of 4
	5:1	**1:1**	**1:0**		
Tigoni X 395438.1	0.14*****	277.44	30.72	Duplex (Ry Ry ry ry)	2
Tigoni X 394905.8	2.31*****	201.84	52.92	Duplex (Ry Ry ry ry)	2
Tigoni X 394904.17	46.84	54.00	147.00	Simplex(Ry ry ry ry), Nulliplex (ry ry ry ry) or Duplex	1 or 2
Tigoni X 395196.4	32.53	552.96	0.48*****	Quadruplex (Ry Ry Ry Ry) or Triplex (Ry Ry Ry ry)	3 or 4
Tigoni X 396286.6	0.0012*****	261.36	34.68	Duplex (Ry Ry ry ry)	2
Tigoni X 396286.7	0.0063*****	261.36	34.68	Duplex (Ry Ry ry ry)	2
Tigoni X 394903.3	9.25*******	150.00	75.00	Duplex (Ry Ry ry ry)	2
Tigoni X Tigoni	948	586	1986	Nulliplex (ry ry ry ry)	0
Tabulated X^2 value at (P=0.05) and at 1 degrees of freedom is 3.84					

* Significant at (P=0.05)
** Significant at (P=0.01)
*** Significant at (P=0.001)
DAI Days after inoculation

DISCUSSIONS

Variations in temperature at the time of cross breeding negatively affect the breeder since it limits the number of seeds and seedlings for screening. In this experiment, a low temperature range of 11-19◦C was found to be most favourable for cross breeding potato while higher temperatures reduced the number of successful crosses. Studies in the past indicate that, pollen germination and pollen tube growth occur at a favourable low temperature and reduces when temperatures are increased (Burton, 1989; Sleper and Poehman, 2006; Roy, 2007). In order to achieve the highest number of successful crosses breeders in developed countries conduct their crosses under conditions (facilities) where suitable temperatures are maintained (Acquaah, 2007). However in developing countries such facilities are rarely available and to breed new varieties one has to conduct crosses in the field during seasons when temperatures are most favourable.

The 1:0 segregation ratio obtained for 395196.4 in this study, implied that when such a parent is used in a breeding program, there would be no need for disease inoculation to eliminate susceptible genotypes. This type of parent either has triplex (Ry Ry Ry ry) or quadruplex (Ry Ry Ry Ry) genes for PVY and PVX resistance (Wastie *et al*., 1992). This is the highest gene dosage which is expected to give 100% of resistant progenies when crossed with a susceptible variety. The parents for breeding PVY and PVX resistance are chosen based on the number of these genes to increase the number of resistant progenies and to reduce the number of progenies eliminated during preliminary screening using sap innoculation (Cadman, 1942; Mendoza *et al.,* 1996). Progenies of (Tigoni X 395438.1), (Tigoni X 396286.7) and (Tigoni X 396286.6), which significantly (P = 0.05) fitted 5:1 segregation ratio (resistant: susceptible progenies) implied that the virus resistant parents (395438.1, 396286.6 and 396286.7) used for this cross were duplex (Ry Ry ry ry) for PVY and PVX resistance genes. To increase the number of PVY and PVX resistance genes, recurrent mass selection is done on selected varieties/clones with desirable combination of virus resistance (Burton, 1989; Acquaah, 2007).

CONCLUSIONS

At KARI, Tigoni in Kenya, low temperatures of 11-19°C found by this study as most favourable is mostly experienced in June/July and October/November and for optimum successful cross pollination of potato in the field, breeders have to coincide flowering of the parental varieties for crossing with such cool seasons. The best PVY and PVX resistant parent identified (395196.4) can be used for breeding PVY and PVX resistance in addition to other market demanded traits of the Kenyan potato industry. This clone can also be evaluated further to identify if it has good yield performance in different agro ecological zones and eventual release. Out of the 8400 seedlings screened in this study, 81 % or 6804 resistant seedlings can be evaluated further to identify other market demanded characteristics in addition to PVY and PVX resistance.

ACKNOWLEDGEMENTS

Special thanks to KARI Tigoni and CIP, Nairobi office for facilitating this research work and for providing the research materials.

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