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Identification of elite potato genotypes possessing multiple disease resistance genes through molecular approaches

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ABSTRACT

World potato productivity including India was almost stagnant during the last two decades largely due to yield losses by biotic and abiotic stresses. Among biotic stresses, late blight, viruses and nematodes are the most devastating. Varieties resistant to individual stress have been deployed but the production remained limited because of other biotic stresses affecting the crop. Potato cultivars having multiple disease resistance are urgently required to boost production. Resistance to late blight is both qualitative and quantitative while extreme resistance to PVY can be imparted by the single dominant genes Ry_{adg} and Ry_{sto}. Likewise resistance to potato cyst nematode is mainly imparted by the single dominant *H1* and *Gro1-4* genes. All these genes have been mapped and tightly linked molecular markers are available to perform marker-assisted selection (MAS). In the present study 126 parental clones were characterized for the presence of genes for resistance to late blight (*R1*, *R2*, *R3a*), PVY (Ry_{adg}, Ry_{sto}) and potato cyst nematodes (*H1*, *HC_QRL* and *Gro1-4*) using molecular markers. The same clones were evaluated for disease resistance with standard phenotypic assays. Fourteen elite potato genotypes possessing multiple disease resistance genes were identified by means of linked molecular markers and their resistances were confirmed through phenotypic screening methods. These genotypes can be exploited as parents for hybridization to expedite the potato resistance breeding programmes.

1. Introduction

Potato (*Solanum tuberosum* L.) is a member of the *Solanaceae*, an economically important plant family. Potato is after the grains, the world's number one food crop with global production reaching a record of 374 million tonnes in 2011 (http://faostat.fao.org/site/339/default.aspx). Today, potatoes are grown in about 150 countries ranging from latitudes 23° N to 57° N and at altitudes from sea level to 4000 amsl. The top three potato producers of the world, China, India and the Russian Federation together contribute about 43% of the global potato production. A yield plateau has been reached for the potato crop in India (Pandit and Chandran, 2011) indicating that the potato varieties developed during the last 50 years did not find substantial favour with the growers. The world potato productivity also did not substantially increase during the last 20 years, though an increase from 14.6 t/ha (1991) to 17.8 t/ha (2010) was observed (http://faostat.fao.org/site/339/default.aspx). Gopal and Oyama (2005) stated that stagnation of the potential yield of potato commercial cultivars is primarily due to their narrow genetic base. Hence, to ensure food security, buffer stocking and exportable surplus, high yielding, multiple disease resistant potato varieties are required (Douglas and Halpin, 2010; Joseph et al., 2011).

Among the various biotic and abiotic stresses affecting potato production, three biotic stresses i.e. late blight, viruses and cyst nematodes constitute major threats. Late blight caused by the oomycete, *Phytophthora infestans* is the most important disease of potato causing severe crop damage worldwide. It causes losses over US $ 3.25 billion in developing countries while in India, it inflicts

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losses up to 15% annually amounting to 0.5 billion US $ (Bhat et al., 2008). Since early in the last century 11 single genes for resistance (*R* genes) to late blight have been introgressed into the cultivated potato from *S. demissum*. This type of resistance, often called vertical resistance, was rapidly overcome in the field by new strains of *P. infestans*, and thus breeders lost interest in single gene resistance (Wastie, 1991). Now-a-days there is renewed interest in this type of resistance as *R* genes, although defeated, can still increase the level of quantitative or field resistance, which is considered more durable (Stewart et al., 2003; Gebhardt et al., 2004). Co-localization of some *R* genes with quantitative resistance loci (QRL) by Gebhardt and Valkonen (2001) suggests that major and minor gene resistance might be in part similar at the molecular level. Most of the *R* genes introgressed from *S. demissum* have been mapped and four of them *R1*, *R2*, *R3a* and *R3b* have been cloned and sequenced (Ballvora et al., 2002; Lokossou et al., 2009; Huang et al., 2005; Li et al., 2011). Many other *R* genes conferring resistance against *P. infestans* have been mapped from wild species including RB/*Rpiblb1*, *Rpi-blb2*, *Rpi-blb3*, *Rpi-bt1* and *Rpi-abpt* (*S. bulbocastanum*); *Rpi-bst1* (*S. brachistotrichum*); *Rpi-edn1.1* (*S. edinense*); *Rpi-hjt1.1*, *Rpi-hjt1.2* and *Rpi-hjt1.3* (*S. hjertingii*); *Rpi-mcd1* (*S. microdontum*); *Rpi-snk1.1* and *Rpi-snk1.2* (*S. schenckii*); *Rpi-ver1* (*S. verrucosum*); *Rpi-pnt1* (*S. pinnatisectum*); *Rpi-sto1* and *Rpi-sto2* (*S. stoloniferum*); *Rpi-pta1* (*S. papita*); *Rpi-plt1* (*S. polytrichon*); *Rpi-mcq1* from *S. mochiquense*; *Rpi-phu1* from *S. phureja*; *Rpi-vnt1.1*, *Rpi-vnt1.2*, *Rpi-vnt1.3* (*S. venturii*); *Rpi-dlc1* from (*S. dulcamara*); *Rpi-ber1* and *Rpi-ber2* (*S. berthaultii*); *Rpi-avl1* (*S. avilesi*); *Rpi-cap1* (*S. capsicibaccatum*); and *Rpi-qum1 S. circaeifolium spp. quimense.* Among these about 20 functional late blight *R* genes have also been cloned so far (Kim et al., 2012). As a result of this work molecular markers either tightly linked to these genes or located within the resistance gene itself have been developed, which can be useful for undertaking marker assisted selection (MAS).

The potato crop is also threatened by a number of viruses which are omnipresent (Jeffries, 1998). The most damaging viral diseases are caused by *Potato Leaf Roll Virus* (PLRV), *Potato Virus Y* (PVY) and *Potato Virus X* (PVX). Currently, PVY causes the most damage and surpassed PLRV in numbers, with yield losses up to 80% (Daniels and Pereira, 2004). Three main strains of PVY are known to infect a wide range of potato cultivars viz., PVY^{C}, PVY^{N} and PVY^{O}, though further PVY strains have emerged due to mutation or recombination among these strains, resulting in more aggressive strains like PVY^{NTN}. The symptoms produced by PVY in potato foliage vary according to the virus strain and the cultivar. PVY^{C} causes a mosaic pattern while PVY^{N} leads to leaf necrosis in tobacco and soft mosaic symptoms in potato foliage. Necrotic rings are produced by PVY^{NTN} and severe necrosis can lead to death of the susceptible plants. PVY^{O} is the most common strain in India, causing light and dark mosaic patterns in the leaves. Further spreading of the virus may lead to vein burning and sometimes necrotic “ringlets” on the leaves.

In India more than 90% of potatoes are grown under sub-tropical conditions that favour the proliferation of viruses because of the congenial conditions prevalent for their insect vectors. This fact makes it difficult to produce virus free healthy seed potatoes. The situation is expected to deteriorate further due to global warming that may result in fewer days available for seed potato production. Hence, breeding virus resistant cultivars as advocated by Ross (1986) needs to be taken up on priority to boost the seed production programmes in the developing world. The strain-specific resistance is controlled by the N_Y resistance gene while the extreme resistance is controlled by R_Y gene. The single dominant gene Ry_{adg} from *S. tuberosum* group *andigena* on potato chromosome XI imparts extreme resistance (ER) to PVY (Munoz et al., 1975). The Ry_{adg} gene can be detected in segregating progenies with the diagnostic DNA marker RYSC3 (Kasai et al., 2000). Recently CPRI has developed and registered the elite potato cultivar YY6/3 C-11 with the National Bureau of Plant Genetic Resources (NBPGR), New Delhi (INGR10143). This cultivar possesses Ry_{adg} in triplex (*YYYy*) condition (Kaushik et al., 2013). The independent gene Ry_{sto} on chromosome XII from *S. stoloniferum* also confers extreme resistance to PVY and can be diagnosed by PCR markers (Flis et al., 2005; Song et al., 2005; Song and Schwarzfischer, 2008). Other sources of resistance genes to PVY are detected in wild species of *S. chacoense* (Ry_{chc}, Ny_{chc}), *S. hougasii* (Ry_{hou}), *S. demissum* (Ny_{dms}) by Cockerham (1970) and Valkonen et al. (1994). Two other novel genes Ny_{tbr} and *Ny-1* were identified in *S. tuberosum* Gp. Chilotanum by Celebi-Toprak et al. (2002) and Szajko et al. (2008) respectively.

The potato cyst nematodes (PCN) *Globodera rostochiensis* and *Globodera pallida* are the most important pests feeding on potato roots (Evans and Trudgill, 1992). The symptoms of infection are unspecific and similar to those caused by other biotic or abiotic stresses. Yield losses caused by PCN are estimated up to 30% worldwide (Oerke et al., 1994). Both PCN species are included in the list of quarantine pathogens in many countries (EPPO/CABI, 1992) including India. The wild *Solanum* species mostly exploited in PCN resistance breeding are *S. tuberosum* ssp. *andigena*, *S. vernei* and *S. spegazzini*. PCN resistance has been reported in other wild sources viz., *S. gourlayi*, *S. sparsipilum*, *S. chacoense*, *S. phureja*, *S. demissum*, *S. gourlayi*, *S. microdontum*, *S. sucrense*, *S. tarijense*, *S. acaule*, *S. fendleri*, *S. multidissectum*, *S. oplocense* (Phillips, 1994). A number of PCN resistance genes have been mapped in different potato chromosomes conferring specific and partial resistance. Major genes conferring specific resistance to *G. rostochiensis* are *H1*, *GroVI*, *Gro1*, *Gro1-4* while *Gpa2*, *GpaV* and *GpaXI* confer resistance against *G. pallida* (Milczarek et al., 2011) Besides, several other major and minor QTL offers partial resistance to either of these *Globodera* species. The phenotypic evaluation of resistance to *Globodera* spp. is costly and time consuming. DNA markers can reduce these costs, when their application in breeding programmes is optimized, for example by consecutive screening or by multiplex PCR assays (Gebhardt et al., 2006). Currently available diagnostic markers for the selection of PCN resistant genotypes (Milczarek et al., 2011; Dalamu et al., 2012) are TG689 (Biryukova et al., 2008), Gro1-4 and Gro 1-4-1 (Paal et al., 2004; Gebhardt et al., 2006; Asano et al., 2012), SPUD1636 (Bryan et al., 2002) and the SNP-based marker HC (Sattarzadeh et al., 2006).

In order to increase and sustain potato production, it is imperative to control these diseases effectively. Chemical control of any of these diseases has its own limitations. Therefore, exploiting genetic resistance was and is always the advised and preferred method for their effective management. Molecular markers tightly linked to different resistance genes have the potential to facilitate the precise and efficient selection of resistant cultivars at an early stage, particularly the stacking of two or more different resistance genes in the same genetic background. The availability of DNA-based markers closely linked to resistance genes/QRLs offers the possibility to screen various types of germplasm. Stacking genes for resistance to late blight and nematodes through this approach will extend the life span of new varieties in the South Indian hill regions while varieties combining resistance to late blight and viruses will have great potential in the sub-tropical plains of India. Developing varieties with diverse resistance genes and their strategic deployment in different agro-climatic zones would help in arresting the spread of major diseases in different potato growing areas. Keeping this in view, the objectives of the present study were (i) to identify parental potato lines possessing multiple disease resistance genes to be exploited in potato breeding programmes and (ii) to test the suitability for MAS of several diagnostic markers for disease resistance in Indian breeding materials.