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PROSPECTS OF MITIGATING LATE BLIGHT DISEASE OF POTATO IN NIGERIA THROUGH DEPLOYMENT OF TRIPLE *R* (3*R*) STACKED GENE TRANSGENIC VARIETIES

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Abstract
Potato (*Solanum tuberosum*) is a cherished staple food in Nigeria. Low average yield of about 4.3t/ha obtained locally is partly due to 20-50% yield reduction caused by late blight disease (LBD) (caused by species of oomycetes known as *Phytophthora infestans*). Based on a price of $150 per ton for potato, the cost of late blight disease is estimated at between $42,000,000 – $105,000,000 per annum. Development and deployment of resistant varieties remain the most economic and environmentally sustainable way to control plant diseases. Stacking resistance *R* genes for *Phytophthora* into farmer-preferred varieties from wild relatives by conventional breeding takes decades to realize, especially when working with polyploids and crops suffering from inbreeding depression. Genetic transformation techniques provide a more direct transfer mechanism into existing elite varieties lacking resistance to LBD. The 3*R* biotech potato was developed by the International Potato Center (CIP) by transferring three *R* genes selected from unmodified DNA fragments of *Solanum bulbocastanum,* and *Solanum venturii* into farmers' preferred varieties lacking resistance to LBD. These genes were chosen for their ability to recognise a broad spectrum of strains of *P. infestans*. The efficacy and safe use of 3*R* Potato have been demonstrated in Uganda and can be scaled out in Nigeria to mitigate the threat posed by LBD. Regulatory approvals for confined field trials of lead events, environmental and commercial release, robust extension and adoption of prospective 3*R* biotech potato varieties by relevant stakeholders will contribute significantly to food security in Nigeria. The prospects are brightened by extant policy environment which appears conducive for obtaining the required regulatory approvals for trial and deployment of potato varieties stacked with 3 *R* resistant genes.

Keywords: 3*R* biotech potato, Late blight disease, Potato, *Phytophthora infestans*

Introduction

Potato (*Solanium tuberosum*), a member of the family *solanaceae*, is a cherished staple food in Nigeria. It is an annual herbaceous tuber crop that is conventionally propagated by tubers. Potato was brought into Nigeria by European tin miners and became established in the cool mid altitudes of Jos plateau as the then British colonial government encouraged its production to enable it feed her soldiers during the first and second world wars (Okonkwo *et al*., 2009). Late blight disease (LBD) was found on potato plants on the Jos Plateau of Nigeria as far back as 1971 (Erinle and Quinn, 1980). Nigeria is the largest producer of potato in the West African sub-region. Production estimate for potato in Nigeria is 1.4 million metric tonnes from 329,061 hectares in 2019 (FAOSTAT, 2019). Average yields of about 4.3 t/ha obtained for potato is far below global average of about 21.4 t/ha (FAOSTAT, *ibid*).

Potato tubers contain a higher amount of carbohydrate, and protein per hectare compared to cereals and is a good source of vitamin C, B_{12}, potassium and fibres (Ghislain *et al*., 2021). Potato contributes significantly to food security and sustenance of livelihoods of subsistent farmers, especially on the highlands where its growth can be economically sustained. Over the years, potato yields have also shown no appreciable increase despite various efforts by government and farmers to increase production (Figure 1). Reasons adduced for low potato yields include; use of poor quality seed (Okonkwo *et al*., 2001), frequent pest and disease out breaks, poor farmer knowledge of appropriate disease and pest management practices (Plaisier *et al*., 2019), lack of knowledge about best agricultural practices (Ayuba, *et al*., 2014) and low input. These low yields are also partly due to 20-50% yield reduction caused by late blight disease (LBD). Late blight disease (LBD) of potato is caused by *Phytophthora infestans* (Mont.) de Bary. The pathogen belongs to a fungus-like group of ubiquitous organisms known as oomycetes (water moulds). In addition to leaf blights which cause serious yield reduction, LBD has been reported to cause serious post-harvest decay of tubers in storage (Johnson, 2008).

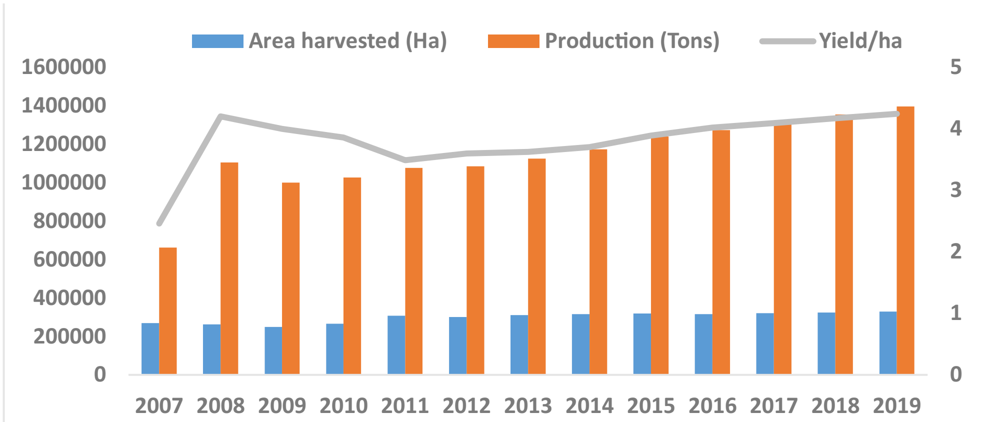

Figure 1: Potato production estimates for Nigeria: 2007 to 2019
Source: FAOSTAT, 2019

Late blight disease is of great economic significance. Based on a price of $150 per ton for potato, the cost of late blight disease is estimated at between $42,000,000 – $105,000,000 per annum. The yield decline and the attendant financial loss caused by LBD reduces food availability, increases food prices and poses danger to income and livelihoods of rural households. Farmers in Nigeria currently use limited amounts of fungicides, partially resistant varieties and a resort to early planting to control LBD with limited success (Amadi *et al*., 2009; Okonkwo *et al*., 2009; Lenka *et al*., 2010). Fungicides have negative impacts on the environment in particular, and the soil eco-systems in general. They are also expensive and sometimes not available. Development and deployment of resistant varieties remain the most economic and environmentally sustainable way to control plant diseases (Singh *et al.*, 2012).

Plant resistance and pathogen virulence have been co-evolving up till now (Zhu *et al*., 2012). Most resistance genes only work against a subset of *P. infestans* isolates, since effective plant disease resistance only results when the pathogen expresses a RXLR effector gene that matches the corresponding plant resistance (R) gene. Effector-R gene interactions trigger a range of plant defences, such as the production of compounds toxic to the pathogen (CIP, 2019; Ghislain, *et al*., 2019). Stacking resistance *R* genes for *Phytophthora* into farmer preferred varieties from wild relatives by conventional breeding is virtually impossible due to the genetic drag of negative alleles from wild species which is difficult to eliminate in an out-breeding tetraploid crop (Ghislain, *et al*., 2019; 2021). It took 45 years to transfer a single resistance *R* gene from *Solanum bulbocastanum* into a modern variety by conventional breeding (Haverkort *et al*., 2009).

Genetic transformation techniques provide a more direct transfer mechanism into existing elite varieties lacking resistance to LBD**.** Techniques like gene guns and Agrobacterium mediation enable the transfer of specific genes coding for proteins that produce specific traits like disease resistance to be transferred directly into the genome of receptor plants without the encumbrance of other unwanted genes (Zhu *et al*., 2012; Haesaert *et al*., 2015). Thus, *R* genes can be introduced simultaneously by genetic engineering into an existing variety without altering any of its properties, except reducing its susceptibility to LB (Zhu *et al*., 2012; Haesaert *et al*., 2015). Resistance conferred by individual resistance genes (*Rpi*) are quickly overcome as the pathogen adapts by mutating their effectors, necessitating the need to stack up multiple *R* genes for broad spectrum resistance in the crop in order to confer durable resistance. Douglas and Halpin (2010) suggested three different transformation methods can be used for *R* gene stacking: (1) transformation of one or two *R* genes followed by re-transformation of a selected, well performing, resistant transformant with additional *R* genes; (2) in one step via co-transformation, by using mixed Agrobacterium strains containing unlinked R genes in two or more vectors; (3) with multiple *R* genes in one vector.

In this paper we review the development and field evaluation of the triple – gene resistance to LBD, and the prospects of mitigating the potato disease in Nigeria through the introduction of the broad spectrum *R* genes for resistance into our elite cultivars, and the impact such intervention can have on food security and livelihood of rural households in Nigeria.

Development and Preliminary Evaluation of 3 R Biotech Potato

In order to achieve the stacking of 3*R* resistance genes in elite varieties, three genes reported to confer broad-spectrum resistance against a wide range of *P. infestans* races (Pel *et al*., 2009; Song *et al*., 2003; Van der Vossen *et al*., 2005) were selected. Two of these genes; *RB* and *Rpi-blb2* came from *Solanum bulbacastum*, while, the third (*Rpi-vnt1*) came from *Solanum venturi* (Magembe *et al*., 2018). Both species are wild relatives of potato (*Solanum tuberosum*). Resistance gene *RB* was cloned after long range PCR amplification. The *Rpi-blb2* and *Rpi-vnt1* genes were synthesized using the DNA sequence deposited in GenBank. The three *R* genes were cloned as genomic fragments (no changes from the original DNA sequence) into a plant transformation vector (pCIP99) carrying the selectable marker gene (*nptII*), which confer resistance to kanamycin (Figure 2) (Magembe *et al*., 2018). Gene transfer was *Agrobacterium tumefacien*-mediated. Agro-infection with the vector pCIP99 bearing the 3 *R* gene stack produced transgenic events from the elite varieties (Magembe *et al*., 2018).

Preliminary evaluation of the isolated events involved an initial screening on media with kanamycin. Those regenerates growing well on kanamycin-media were then tested by PCR for absence of vector backbone sequence, for presence of the 3*R* genes, and of their completeness. These tests were important as the events were to be considered in the future for commercial release (Ghislain *et al*., 2019). Those transgenic events without backbone vector sequence and complete T-DNA were then analysed for the number of insertion sites estimated by Southern blotting or digital PCR. Those transgenic events with single insert copy are then tested for resistance to LB using detached leaf bioassays. Finally, the insertion region was sequenced using target capture sequencing or Xdrop sequencing. The transgenic events with all 3*R* genes intact and complete, inserted preferably at a known non-coding location selected as the candidate lead transgenic events to go for field trials and regulatory testing for possible commercial release (Ghislain *et al*., 2019).

The 3R Biotech Potato

The 3*R* biotech potato was developed by the International Potato Center (CIP) by transferring three *R* genes selected from unmodified DNA fragments of *Solanum bulbocastanum* and *Solanum venturii* into farmers' preferred varieties lacking resistance to LBD (Magembe *et al*., 2018). The durability and resilience the LBD resistance of 3*R* biotech potato is buttressed not only by the broad spectrum nature of the *R* genes but also by the fact that no known isolates of *P. infestans* were virulent on both wild species, and none had been identified that were able to overcome all three *R* genes (Vleeshouwers *et al*., 2011: Vleeshouwers and Oliver, 2014). The stacking of the three *R* genes has a positive effect on LBD resistance compared to a single *R* gene increasing the frequency of the desirable phenotype (extreme resistance) from 3 - 9% with single *R* genes to 75% when all three *R* genes were stacked (Ghislain, *et al*., 2019). Three hundred and thirty-one Desiree, 77 'Victoria/Asante', 102 'Tigoni' and 86 'Shangi' transgenic events (TE) were produced with 3*R* gene stack (Ghislain, *et al*., 2014; Magembe *et al*., 2018). Yields of two transgenic events from 'Desiree' and 'Victoria' grown without fungicide to reflect small-scale farm holders were estimated to be 29 and 45t/ha respectively (Figure 3) (Ghislain, *et al*., 2019). This represents a three to four-fold increase over the national average. Thus, these late blight resistant potato varieties, which are the farmers' preferred varieties, have the potential to bring significant income to smallholder farmers in sub-Saharan Africa, if rapidly adopted (Ghislain, *et al*., 2021). The 3*R* potatoes have been developed and field-tested over the last decade in a wide diversity of environments in Uganda (Figure 4), the Netherlands, Belgium, Ireland, United Kingdom, Sweden, U.S., Indonesia and Bangladesh. At all locations, the transgenic potatoes were grown without fungicides and better yield without any negative impact

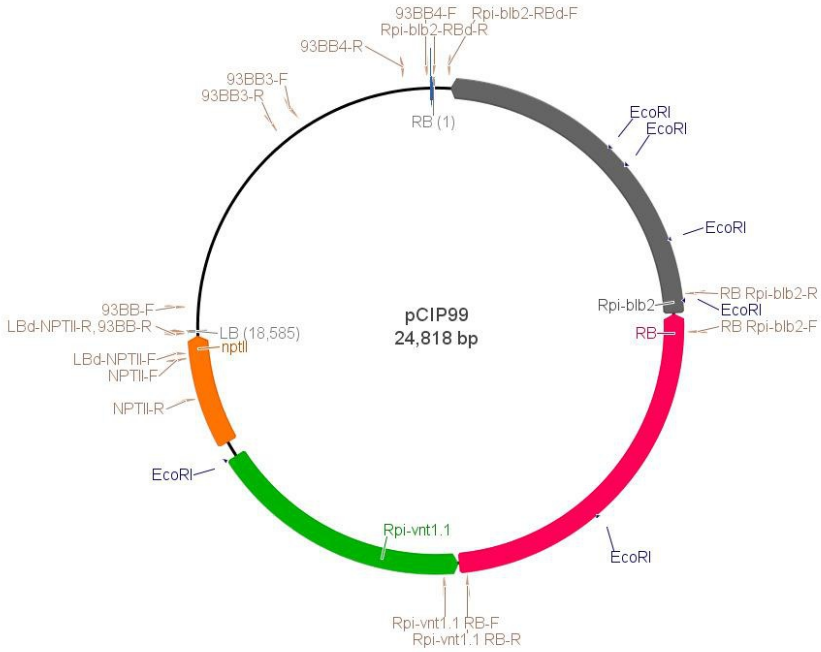

Figure 2: pCIP99 3R gene stack construct bearing the three R genes (Rpi-blb2, RB, and Rpi-vnt1.1) and the selectable marker gene (nptII) used for transformation of potato
Source: Magembe et al. (2018)

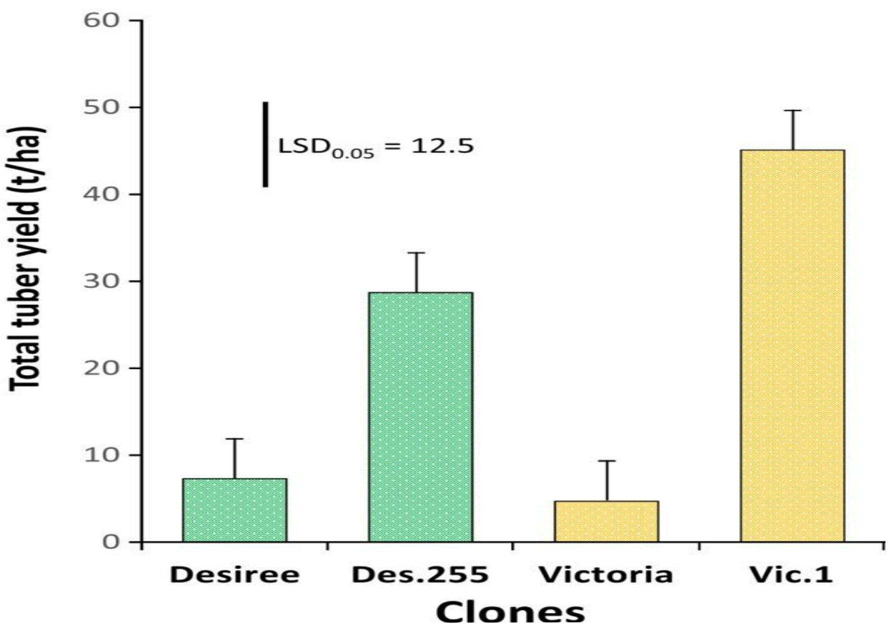

Figure 3: Total yield of non-transgenic and transgenic 'Desiree' and 'Victoria' at KaZARDI, Southwest Uganda in the second cropping season of 2016 (CFT-4). Des 255 and Vic.1 are the transgenic events from 'Desiree' and 'Victoria', respectively. The error bar is measured and nipped from the y-axis. Value of error bar determined from LSD test at 0.05 probability is 12.47
Source: (Ghislain et al., 2019)

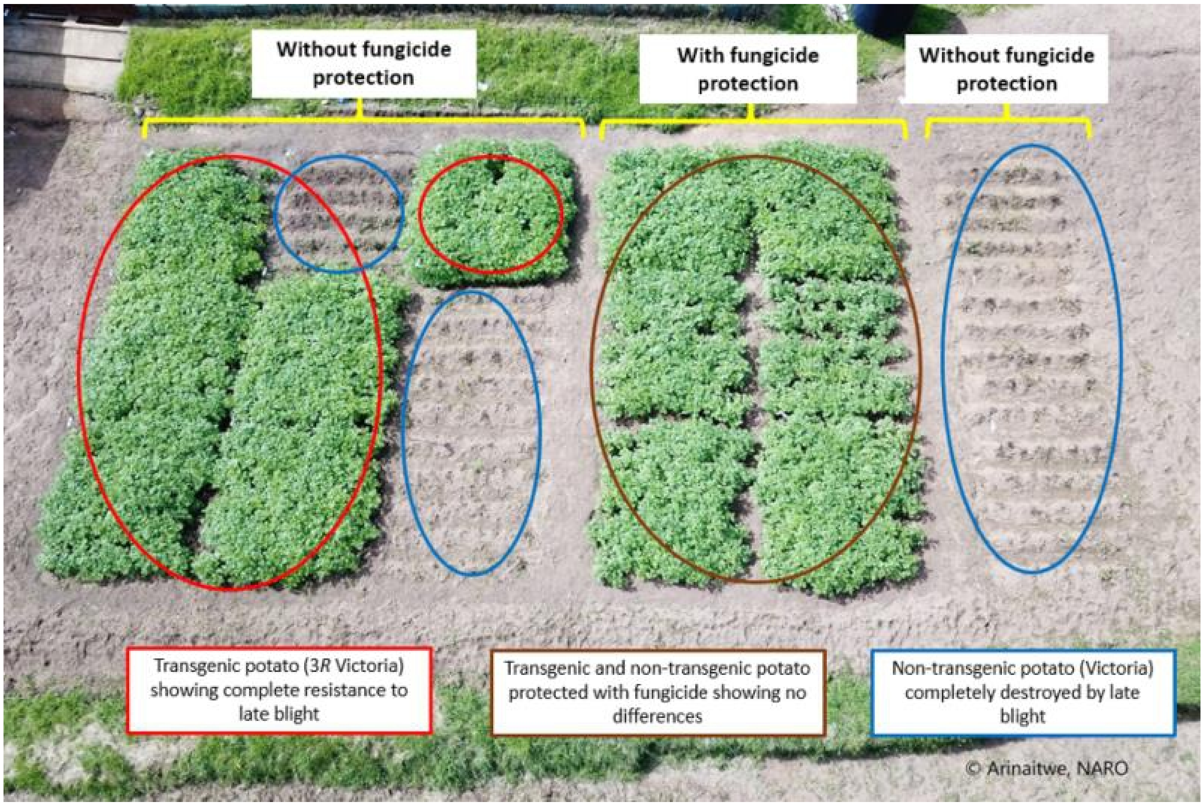

Figure 4: Devastation by late blight disease – Confined field trial at Kachwekano Research Station in Uganda in 2019 under natural infestation by late blight with plots planted with either 3R Victoria or Victoria
Source: Ghislain, et al. (2021)

Safety of 3R Biotech Potato

Yield and morphological characteristics of the tubers of 3*R* biotech potatoes were not significantly different from those obtained from the non-transgenic varieties protected by fungicides (Ghislain, *et al*., 2019). Other results showing that R-gene-mediated resistance does not affect yield or tuber characteristics have been previously reported (Halterman *et al*., 2008; Jones *et al*., 2014). Tuber samples from the transgenic potato and the non-transgenic variety (parental line) were analysed for the essential five components recommended by the OECD. Moisture, sugars (especially reducing sugars), vitamin C, ash, protein, and glycoalkaloid contents were mostly identical and all values were within the range reported in the literature for potato tubers (OECD 2015; AFSI 2019). Field studies of transgenic potatoes expressing *R* proteins in Netherlands, Ireland and Uganda have not shown differences in arthropod abundance compared to conventional potato lines in different locations over two to five seasons (Ghislain, *et al*., 2021). This suggests no negative impact of these biotech products on the environment.

Potential of Scaling out of CIP 3R Biotech Potato in Nigeria

The efficacy and safe use of 3*R* potato have been demonstrated in Uganda and can be scaled out in Nigeria where LBD remains the most limiting disease of potato. Estimated yields of 29t/ha for Desire and 45t/ha for Victoria by 3*R* potato represents very highly significant increase over the national average of 4.3t/ha. The replacement of the existing LB susceptible varieties by resistant ones would radically change the lives of many potato growers by increasing incomes and reducing health risks due to exposure to fungicides. Variety 'Desiree', one of the transformed elite varieties, is one of the varieties already grown in Nigeria, so acceptability and adoption by farmers is not envisaged to be a problem. In addition, only one genotype of *P. infestans* Eu_33_A2 has been reported in Nigeria (Nnadi *et al*., 2019). The low diversity in the local pathogen population and the expression of the cognate avirulence effector genes of the 3*R* gene stack, suggest that the resistance to late blight conferred by 3*R* genes may be long-lasting in Nigeria. Also, only the *P. infestans* A1 mating type is present in Nigeria, so no sexual recombination is expected to occur to diversify the population. However, the pathogen population will need to be monitored seasonally, at least in the regions where the 3*R*-gene potato will be released, for the possible emergence of new strains. Regulatory and policy environment in Nigeria appears clement for a possible release of biotech potato. A pointer to this is the recent release for commercial use of cotton and *Bt* cowpea in 2019. Regulatory approvals for confined field trials by the relevant agency are most likely to be obtained. Identification of candidate varieties and their environmental and commercial release will make 3*R* biotech potato available for farmers' use. However, considering the perception of some segments of the society, robust sensitization through different avenues, channels and media will be required to create the necessary awareness to fast-track extension and adoption of prospective 3*R* biotech potato varieties by relevant stakeholders. Such techniques include; individual methods (like farm and home visits), group methods (such as group discussions), exhibitions, tours and field trips, demonstration plots, farmer field days, and mass contact methods (such as radio, television, cinema, public address systems, newspapers, posters, leaflets, bulletins, magazines and other printed materials used to reach large audience) (Ekerete and Ekanem, 2016). Ultimately, for sustainability, our consumer and farmer-preferred varieties such as Nicola, Bertita, Lady christyl, Diamant, RC767-2 and Marabel, need to be transformed and stacked with 3*R* genes to enhance their resistance to LBD. This is quite feasible since potato is known to respond well to transformation using Agrobacterium.

Conclusion

The prospect of scaling out 3*R* Biotech Potato to mitigate the adverse effect of late blight disease of potato in Nigeria is exciting. But even more exciting are the prospects of adapting this model to stack up LBD resistance genes into our elite cultivars of potato. Extant regulatory and policy environment appears conducive for this biotech solution to our *Phytophthora* problems in potato.

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