Induced mutagenesis: an underutilised component in the integrated management of aphid pests in Sub-Saharan Africa

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Induced mutagenesis: An underutilised component in the integrated management of aphid pests in Sub-Saharan Africa

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Abstract

Aphids (Hemiptera: Aphididae) are important agricultural pests in sub-Saharan Africa. These pests are primarily controlled by the use of synthetic insecticides, which has consequently led to the emergence of insecticide-resistant aphid populations as well as negative impacts on non-target organisms. Resistant crop varieties offer a sustainable approach to manage aphids. Despite regions of sub-Saharan Africa suffering greater crop losses due to pests, there is only limited availability of genetic engineering and other modern plant breeding technologies. Here we consider whether induced mutagenesis can contribute to the sustainable management of aphid pests or whether the lack of research in this area reflects the limitations of this approach.

Keywords: genetic variation, genetic improvement, mutant, plant breeding, resistant varieties

1.0 Introduction

Nearly one billion people are food insecure in Sub-Saharan Africa today (Botha et al., 2020). Predictions indicate that in the absence of effective mitigation measures, reliable access to sufficient, affordable and nutritious food is likely to deteriorate further in the next 50 years. Global crop loss estimates per crop of 21.5, 30.0, 22.6, 17.2 and 21.4 % are caused by pests and diseases on wheat, rice, maize, potato and soybean respectively (Savary et al., 2019). However, these overall estimates mask the very large differences in crop losses among different food insecurity hotspots such as Sub-Saharan Africa. Aphids (Hemiptera: Aphididae) are amongst the most damaging invertebrate pests affecting crop productivity (Leybourne et al., 2019). Aphid management has, like for many other crop pests, predominantly relied on the application of synthetic chemical insecticides (Roubos et al*.,* 2014). While

use of such products has greatly enhanced crop productivity during the last century (Naik et al., 2019), issues of resistance due to their overuse as well as their negative effects on human and environmental health are now widely recognised (Kim et al., 2017). Public concerns regarding pesticide use, particularly chemical residues on harvested crops and their products, have also increased significantly during the past few decades (Schaub et al., 2020). These issues have led to increasingly restrictive legislation on insecticide use and, consequently, a diminishing portfolio of effective active ingredients available to growers for invertebrate pest management.

Resistant crop varieties offer an environmentally sustainable management option for aphids (Pertot et al., 2017). The rapid emergence of plant resistance-breaking aphid biotypes, however, necessitates a regular supply of varieties with new forms of resistance. Despite regions of Sub-Saharan Africa suffering greater crop losses due to pests, availability of genetic engineering and other modern plant breeding technologies (i.e., targeted mutagenesis) are less available (Botha et al., 2020), likely due to the lagging pace in technology, inadequate research funding schemes as well as hesitance of policymakers to establish biosafety laws (Agbowuro et al., 2021; Botha et al., 2020). By contrast, induced mutagenesis is a cost effective, widely accepted tool used for generating genetic variation to abiotic (i.e., drought tolerance) and biotic (i.e., pest resistance) stresses (Singh et al., 2006).

Mutagenesis refers to heritable alterations in the genetic material that gives rise to individuals with modified phenotypic traits and provides a source of unique germplasm to facilitate crop improvement (Box 1). Such genetic alterations can be induced by exposing a plant or its propagules to physical or chemical materials with mutagenic properties (Viana et al., 2019). Mutations in the DNA are described based on the alteration of gene functions (Mba, 2013). The common types of mutation induced in the DNA that are relevant to crop improvement include: single base substitutions, point mutations, insertions and deletions (Mba, 2013) (Table 1). Induced mutagenesis has played a key role in the genetic improvement of crops for decades, with the joint Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA) database containing 3,275 mutant crop varieties derived from 225 plant species (FAO/IAEA, 2019). Mutation derived varieties are now cultivated in most parts of the world, including: Asia, Europe and North America (Horn et al., 2015). Use of induced mutagenesis in plant breeding programs has increased in recent years due to the development of efficient and cost effective mutation-detection techniques such as Targeting Induced Local Lesions in Genomes (TILLING) (Viana et al., 2019). As a technique, induced mutagenesis has been widely adopted by plant breeders targeting pathogen resistance and other abiotic stresses (Oladosu et al., 2016). Few studies, however, have considered using this approach to develop aphid resistant plant varieties. Reasons for why induced mutagenesis should be considered as a means of developing aphid resistant crops in Sub-Saharan Africa are here classified into the following topics: (1) those related to technological issues (i.e., accessibility and legislation) associated with other accelerated approaches to plant breeding, (2) similarities between aphid and pathogen resistance mechanisms, (3) aphid and pathogen resistant genes often being found close together on chromosomes, and (4) improved screening of mutagenised plant population.

2.0 Prospects for the use of induced mutagenesis as a tool for developing aphid resistant crop varieties

2.1 Technological issues associated with other accelerated approaches to plant breeding

Several crop improvement technologies such as genetic engineering, marker assisted selection and targeted mutagenesis have been developed and may help to accelerate plant breeding for aphid resistance (Bhattacharya, 2019; Voss-Fels et al., 2019; Wang et al., 2019). Despite the potential that these modern plant breeding tools offer to plant breeding, their practical use in Sub-Saharan Africa is limited. The use of external DNA in genetically engineered crops, for example, has led to strict biosafety regulation for their use in most Sub-Saharan African countries (Zaidi et al., 2019). In countries like Kenya, for example, where use of genetically engineered crops has been approved, environmental exposure as well as trade of these crops and their products is still prohibited (Botha et al., 2020). Sub-Saharan African countries are largely dominated by smallholder farmers with less financial capacity to annually purchase genetically engineered crop seed (Fischer et al., 2015). There have also been concerns regarding perceived potential risks of genetically enginnered crops on domestic agricultural biodiversity (Jacobsen et al., 2013). In contrast to genetic engineering, targeted mutagenesis involves alteration of endogenous genes (Arora and Narula, 2017). Despite the non-integration of external DNA, there is increasing pressure to subject gene-edited crops to the same regulations as crops that are genetically engineered, perhaps due to uncertainty around the intended effects of artificially manipulating plants in this way (Callaway, 2018). In addition, the costs associated with new genomic tools, lack of skilled scientific personnel and laboratories hinder the use of modern molecular approaches to plant breeding in Sub-Saharan Africa (Botha et al., 2020). In comparison to these modern plant breeding tools, induced mutagenesis is more widely used and accepted as a breeding tool with a long history of safe use. The non-involvement or use of external DNA in induced mutagenesis exempts mutation derived plants from the often expensive and long regulatory procedures that genetically engineered plants are subjected to (Mba, 2013). This simplified regulatory regime for release of mutant varieties coupled with the robustness, simplicity and low operation costs make induced mutagenesis especially suitable for countries in Sub-Saharan Africa (Mba, 2013).

2.2 Similarities between aphid and pathogen resistance mechanisms

Based on the partial overlap between plant-resistance mechanisms against aphids and microbial pathogens (Kaloshian and Walling, 2005), the production of genetic material with disease resistance provides hope for developing aphid resistant cultivars through induced mutagenesis. Plants recognise pathogen-effector proteins (e.g., flagellin, peptidoglycan, lipopolysaccharides in bacteria and chitins in fungi) using receptors on cell walls that trigger defence responses known as pathogen associated molecular pattern (PAMP)-triggered immunity (PTI) (Chisholm et al., 2006). Pathogens, however, have evolved effector proteins that can suppress PTI in plants (Louis et al., 2012). In response, plants have equally evolved additional *R* proteins that can recognise these pathogen effectors leading to effector-triggered immunity (ETI) (Chisholm et al., 2006). Plants perceive and recognize aphids by detecting specific

effector proteins in aphid saliva (e.g., pectinases, cellulases) in a similar way to the detection of pathogens (Dogimont et al., 2010). During feeding, apids inject watery saliva containing proteins and other metabolites into sieve elements (Louis et al., 2012). The protein molecules in aphid saliva are similar to pathogen associated molecular patterns (PAMPs) that are recognised by pattern recognition receptors (PRRs) in plants to trigger PTI (Rodriguez and Bos, 2013). To counteract PTI, aphids deliver effector proteins in their host plant to suppress this defense promoting effector-triggered susceptibility (ETS) (Jaouannet et al., 2014). In return, some plant species may carry receptors or *R* proteins that can recognise effectors in aphid saliva leading to ETI in plants (Jaouannet et al., 2014). Detection of pathogens or aphid species both result in activation of the salicylic acid (SA) signalling pathway likely due to the limited physical damaged to foliage during feeding (Züst and Agrawal, 2016). Indeed, there is evidence that aphids are negatively affected by the activation of the SA pathway. For example, mutant genotypes of *Arabidopsis thaliana* (L.) Heynh with increased SA signalling have been shown to be less susceptible to peach-potato aphid (*Myzus persicae* Sulzer) (Kerchev et al., 2013). Similarly, growth rate and population growth of the potato aphid (*Macrosiphum euphorbiae*) is adversely affected by the SA signaling pathway mediated by the *Mi*-1 gene in tomato (Li et al., 2006). Therefore, it is likely that disease resistant mutants could also resist species of aphid that are vulnerable to the SA signalling pathway.

2.3 Aphid and pathogen resistant genes often being found close together on chromosomes

Aphid and pathogen resistance genes are often clustered on the same region of the chromosomes (Dogimont et al., 2010; Seah et al., 2007; Stewart et al., 2009). In apple (*Malus domestica* Borkh), for example, woolly apple aphid (*Eriosoma lanigerum*) resistance genes (*Er1* and *Er2*), on chromosomes 8 and 17 respectively, are located on the same genomic regions with genes for resistance to powdery mildew (Bus et al., 2008). The *Ra* gene on chromosome 2 that mediates resistance in lettuce against the lettuce root aphid (*Pemphigus bursarius* L.) is clustered together with downy mildew resistance genes on the same chromosome (Christopoulou et al., 2015). Similarly, the potato aphid (*M. euphorbiae*) resistance gene (*Mi*-1) on chromosome 6 in tomato shares the same location (chromosomal region) with disease resistance genes (Seah et al., 2007). Due to this common genomic locale of aphid and pathogen resistance genes, chromosomal alterations due to induced mutagenesis are likely to induce genetic variations for both pathogen and aphid resistance traits.

2.4 Screening of mutagenised plant population

Induced mutagenesis often introduces random changes in the target organism's genome, making it difficult to precisely target specific genes controlling a desired trait. This lack of specificity requires labour intensive screening of large mutant populations (approximately 5,000 to 10,000 genotypes) to optimise chances of finding desirable mutations. To overcome this limitation, techniques such as TILLING (Targeted Induced Local Lesions IN Genomes)

have been developed to enhance the detection of useful mutations in mutagenised plant populations (Penna and Jain, 2017). The TILLING technique combines mutagenesis and polymerase chain reaction (PCR) technology to identify point mutations such as single nucleotide polymorphisms (SNPs) in target genes (Irshad et al., 2020). In particular, TILLING allows for identification of variations in mutant genome providing a criteria for shortlisting mutants with potential aphid resistance to include in phenotypic screening (Viana et al., 2019). This molecular approach for identifying mutations, as opposed to whole plants in conventional screening, makes TILLING a high throughput and cost effective screening method. The improved capability of genomic tools in recent years offers more thorough investigations of gene structure and function in mutant genotypes which could allow for easier identification, introgression and molecular characterisation of durable resistance to aphid pests.

3.0 Discussion and conclusion

Relatively few studies have considered the application of induced mutagenesis to develop aphid resistant cultivars, perhaps because induced mutagenesis may result in loss-of-gene function and produces alleles that are often recessive to wild type plants (Sikora et al., 2011). Additionally, induced mutagenesis may alter only one or a few genes producing only minor changes in amino acid composition. Since durable aphid resistance in crops is often mediated by polygenic dominant alleles (Smith and Chuang, 2014), creating polygenic resistance, therefore, is rare using induced mutagenesis (Mba, 2013). However, there have been some notable success in breeding for aphid resistance. Using induced mutagenesis (γ-irradiation) on banana (*cv*. Lakatan), Cueva et al. (2014) succeeded in developing mutants that were repellent and resistant to colonisation by banana aphid (*Pentalonia nigronervosa* Coquerel). Similarly, Pathak (1991) successfully developed cowpea mutants that were not only repellent but also inhibited survival and reproduction of the cowpea aphid (*Aphis craccivora* Koch). Mutants derived from turnip cultivars were found to be resistant to mustard aphid (*Lipaphis erysimi* Kaltenbach), and this resistance was attributed to the non-waxy leaves on these plants. Using a chemical mutagen, Susrama and Pradnyawathi (2019) succeeded in developing mutants of common bean, cowpea and yardlong bean that showed resistance to the cowpea aphid. Similarly, Zimba et al. (in press) showed that mutant cowpea genotypes developed using gamma irradiation reduced colonisation, feeding and population growth of cowpea aphid. Characterisation of feeding behaviour using electrical penetration graph recording indicated that resistance to cowpea aphid in cowpea mutants was mediated by epidermal and mesophyll-based resistance factors.

Use of induced mutagenesis is, however, associated with several limitations. Treatment of plant material by mutagens invariably kills cells causing a wide range of deformities and other side effects (e.g., sterility) in surviving plants (Mba et al., 2010). These deformities are often inherited even in mutant plants with desirable characteristics (Mba et al., 2010). Potential mutants, therefore, usually require several generations of successive propagations or crossing with other genotypes to exclude undesirable side effects from their genetic background (Mba et al., 2010).

Mutations arising from induced mutagenesis also have random non-target effects in the genome, making it difficult to precisely target specific genes controlling a desired characteristic (Chaudhary et al., 2019). Therefore, induced mutagenesis programmes are usually 'trial and error' undertakings in which finding a mutant genotype with desirable characteristics is not guaranteed (Chaudhary et al., 2019).

The small number of previously reported successes of using induced mutagenesis to produce genotypes with resistance to aphid pests indicate the potential of this approach. Despite this, the far larger number of successes in breeding for disease resistant crops using induced mutagenesis (Busungu et al., 2016; Jung et al., 2005; Oladosu et al., 2016) and the overlap between aphid and pathogen resistance mechanisms indicate that breeding for aphid resistance is a comparatively under exploited use for this technique. This conclusion is further supported by the common location of pathogen and aphid resistant genes on chromosomes. Furthermore, the long history of safe use, low cost of equipment as well as wide acceptability makes induced mutagenesis an important technique that could be exploited further to speed up the delivery of aphid resistant crop varieties in Sub-Saharan Africa. This is emphasised by a current lack of policy frameworks to regulate the use of modern breeding tools in most countries of Sub-Saharan Africa. Although developing aphid resistance using induced mutagenesis is associated with several challenges, this approach provides a practical means through which to develop sustainable management programmes for aphid pests in crops throughout regions such as Sub-Saharan Africa.

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Table 1: Major chemical and physical mutagens used for induction of random mutations in plants

Mutagens	Type of mutation	Mutation derived traits	References
Chemical agents			
Ethyl Methanesulfonate	Guanine alkylation, G/C to A/T transitions or G/C to C/G or G/C to T/A transversions	Plant development and metabolism Abiotic stress tolerance	(Feldman et al., 2017) (Xu et al., 2017)
N-methyl-N-nitrosourea	Guanine and cytosine alkylation, G/C to T/A transitions	Biotic stress tolerance Nutritional quality Yield and quality improvement	(Busungu et al., 2016) (Kim et al., 2018) (Long et al., 2017)
Sodium azide	Generates azidoalanine causing G/C to A/T transitions	Abiotic stress tolerance Nutritional improvement Yield and quality improvement	(Hussain et al., 2012) (Jeng et al., 2012) (Lin et al., 2014)
Colchicine	Chromosome doubling, affects the microtubules promoting symmetric cell division.	Nutritional improvement Abiotic stress tolerance Yield and quality improvement	(Viana et al., 2019) (Tu et al., 2014) (Guo et al., 2017)
Physical agents			
Gamma-Rays	Single nucleotide substitution, inversion and deletion	Plant development and metabolism Abiotic stress tolerance Nutritional improvement	(Smillie et al., 2012) (Song et al., 2012) (Hwang et al., 2014)
Ion Beam Radiation	Point mutation (deletion), inversion, translocation and insertion	Plant development and metabolism Nutritional quality	(Phanchaisri et al., 2007) (Ishikawa et al., 2012)
Fast-Neutron Irradiation	A/T to G/C transition, insertion, inversion, duplication and deletion	Abiotic stress tolerance Biotic stress resistance	(Ruengphayak et al., 2015) (Chern et al., 2016)

Box 1. Illustration of a generalised procedure for induced mutagenesis.

Seed is denoted as 'M_0' before mutagen treatment and 'M_{1-n}' for generations following mutagenesis. 'M' = meotic generation. After mutagenesis, M_1 seed is planted to produce M_1 plants and M_2 seed. Due to heterozygosity of M_1 plants, mutations are not yet visible at this stage. Seed is harvested, bulked and planted to produce M_2 plants and M_3 seed. Mutations begin to appear in the M_2 population due to genetic recombination and segregations which marks the beginning of screening and selection of desired mutants. From M_3 onwards, seed is harvested from individual plants and planted as single plant-progenies to facilitate detailed screening of mutants. Several generations (i.e. M_{3-6}) are required for mutant genotypes to reach homogeneity. Homogenous mutants (i.e. M_{5-6}) with desired traits can be directly used as a variety or as parents in breeding programmes