Gamma irradiation as a tool to produce cowpea (Vigna unguiculate (L.) Walp.) genotypes resistant to aphid pests

by Zimba, K.J., Sohati, P.H., Munyinda, K., Roberts, J.M. and Pope, T.W.

Zimba, K.J., Sohati, P.H., Munyinda, K., Roberts, J.M. and Pope, T.W. (2022) 'Gamma irradiation as a tool to produce cowpea (Vigna unguiculate (L.) Walp.) genotypes resistant to aphid pests', *Arthropod-Plant Interactions*.

07 September 2022

Gamma irradiation as a tool to produce cowpea (*Vigna unguiculate* (L.) Walp.) genotypes resistant to aphid pests

Kennedy J. Zimba [1,2] · Philemon H. Sohati [2] · Kalaluka Munyinda [2] · Joe M. Roberts[1] · Tom W. Pope[1]

[1] Centre for Integrated Pest Management, Agriculture and Environment Department, Harper Adams University, Newport, Shropshire, TF10 8NB, United Kingdom.

[2] School of Agricultural Sciences, Department of Plant Sciences, University of Zambia, Great East Road Campus, Lusaka 10101, Zambia.

Correspondence: zimbakj@gmail.com

Abstract

Cowpea aphid (*Aphis craccivora* Koch.) is an important pest of cowpea. This study aimed to identify aphid resistant cowpea (*Vigna unguiculate* (L.) Walp.) genotypes derived from three susceptible varieties widely grown in Zambia (Bubebe; Lutembwe and Msandile) after mutagenesis by gamma radiation. Eleven genotypes derived in this way were evaluated: six (BB3, BB7, BB8, BB10, BB14 and BBV) from Bubebe, three (LT3, LT4 and LT11) from Lutembwe and two (MS1 and MS10) from Msandile. Aphid resistance was evaluated by recording aphid colony growth, mean relative growth rate (MRGR), intrinsic rate of natural increase (r_m), doubling time (DT) and feeding behaviour when reared on each genotype. Where colony growth was recorded, significantly lower numbers of aphids were recorded on genotypes BB7, LT3, LT4 and LT11 compared to their parents (Bubebe and Lutembwe). Genotypes LT3, LT4 and LT11 also resulted in lower aphid MRGRs, r_m and DT compared to the parent. Slower colony growth, MRGRs, r_m and DT on genotypes LT3, LT4 and LT11 and slower colony growth only on genotype BB7 suggests the presence of mutation derived resistance to cowpea aphid. Characterisation of feeding behaviour on LT3, LT4 and LT11 using electrical penetration graph recording showed that resistance to cowpea aphid is mediated by epidermal and mesophyll-based resistance factors. BB7, LT3, LT4 and LT11 are therefore promising genotypes that should be evaluated further for genetic improvement of cowpea against the cowpea aphid. This study highlights the potential contribution of induced mutagenesis in the integrated management of aphid pests.

Keywords mutagenesis· resistant varieties· colony growth· gamma radiation· genetic variation

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.), also known as black eye pea, is an important leguminous food crop cultivated across Sub-Saharan Africa (SSA) (Boukar et al. 2019). The grain and leaves of this staple crop have a high protein content, approximately 23 and 44 % respectively, providing an excellent source of dietary protein and livestock fodder (Horn et al. 2016; Samireddypalle et al. 2017). Alongside high protein content, cowpea is also a rich source of vitamins, micronutrients and amino acids (Jayathilake et al. 2018), which are extremely low in SSA diets typically dominated by cereals (Okoth et al. 2017). Cowpea consumption is further encouraged due to their high polyphenolic, flavonoids and bioactive peptide levels, which can reduce risk of certain health conditions like obesity or heart disease (Jayathilake et al. 2018). Being drought tolerant, cowpea can be grown in areas with marginal rainfall and limited irrigation potential (Agbicodo et al. 2009). Like most legumes, cowpea is able to fix atmospheric nitrogen through rhizobium symbiosis (Ehlers and Hall 1996) and adapted to grow in nutrient deficient soils (Elowad and Hall 1987). Despite the economic and agronomic importance of cowpea, yields produced by this crop in SSA are low, ranging from 250-350 kg/ha, compared to potential yields of 2000 kg/ha (Boukar et al. 2019). Insect pests, particularly aphids and the viral diseases they transmit, are among major constraints to achieving optimal yields (Saranya et al. 2010).

Cowpea aphid (*Aphis craccivora* Koch.) (Hemiptera: Aphididae), is an economically important cowpea pest in SSA (Pettersson et al. 1998). Aphids damage crops directly by feeding on phloem sap or indirectly through transmission of diseases (Ofuya 1997; Obopile and Ositile 2010). As aphids preferentially feed on seedlings, when plants are at their most vulnerable stage, large populations can reduce plant health (*e.g.*, stunting) through direct feeding (Huynh et al. 2015). Ingestion of large volumes of phloem sap by aphids results in excretion of excess sugars as honeydew (Wilkinson and Douglas 2003), which provides a growth substrate for sooty moulds that reduce photosynthesis (Ouédraogo et al. 2018). The cumulative effect of feeding damage and sooty mould growth is stunting, delayed flowering, abortion of flower buds and plant death (Jackai and Daoust 1986). Cowpea aphid also transmits cowpea aphid-borne virus (CMV), a major disease that causes between 10% and 100% yield losses (Atiri et al. 1986; Taiwo et al. 2007). Cowpea aphid can cause yield losses of up to 50 % in the absence of control measures.

Foliar application of synthetic chemical insecticides, such as cypermethrin, deltamethrin, lambda-cyhalothrin, pirimicarb or thiamethoxam, are often used to reduce cowpea aphid populations (Musenga et al. 2016; Ezeaku et al. 2017; Reddy et al. 2018). However, the majority of SSA cowpea growers are unable to rely on chemical control due to these products often not being affordable or available (Bata et al. 1987; Ofuya 1997). This issue is further compounded by an overreliance on these products causing target organism resistance to certain active ingredients and therefore reducing their efficacy (Chen et al. 2007; Foster et al. 2014). It is also widely accepted that synthetic chemical insecticides can have negative impacts on human health and the environment if not sustainably used within an integrated pest management (IPM) framework (Desneux et al. 2006; James et al. 2016). In Europe, for example, several insecticides have been withdrawn for these reasons, leaving growers with few effective options for aphid management (Holland et al. 2019). There is an urgent need for cost-effective and sustainable alternatives to synthetic insecticides for managing cowpea aphid populations.

Insect resistant crop varieties offer a cost effective and environmentally friendly method for managing aphids in agricultural systems (Stout and Davis 2009). Such varieties may reduce reliance on synthetic pesticides to promote increased biodiversity and natural pest suppression (Pertot et al. 2017). Varietal resistance, however, is often overcome by the emergence of aphid biotypes adapted to survive on resistant plants (Yates and Michel 2018). For example, single dominant genes *Rac-1* (Bata et al. 1987) and *Rac-2* (Ombakho et al. 1987) that conferred resistance in most SSA cowpea cultivars succumbed to resistance-breaking cowpea aphid biotypes (Boukar et al. 2019). To maintain the effectiveness of insect resistant crop varieties, therefore, requires a regular supply of plant material with new sources of aphid resistance (Yates and Michel 2018).

Aphid resistance traits in plants may be classified in to three categories: (i) chemical deterrence to settling, (ii) physical barriers to feeding, and (iii) reduction in palatability (Züst and Agrawal 2016, Nalam et al. 2019). Plant cells on leaf surfaces often harbour lipids and secondary metabolites that may release aphid deterrent volatiles (Nalam et al. 2019). Trichomes on plant surfaces provide a physical barrier to aphid movement and feeding (Jaouannet et al. 2014). Plants may contain compounds such as protease inhibitors and lectins which reduce palatability of phloem sap to aphids. Lectins bind to carbohydrates in the midgut of insects, interfering with their digestion processes and consequently reducing the performance of aphids (Chougule and Bonning 2012). Protease inhibitors interfere with protease function in herbivorous insects and inhibit protein metabolism (Zhu-Salzman and Zeng 2015). These anti-aphid plant traits may be expressed either constitutively or induced by feeding (Smith and Chuang 2014). To successfully breed aphid resistant cultivars, sources of resistance are needed. Such resistance sources could include wild relatives of crops, germplasm collections or induced mutations (Olasupo et al. 2018).

Mutations can be induced by exposing plant propagules to physical or chemical mutagens that cause genetic changes within the crop and generate different crop phenotypes (Novak and Brunner 1992; Mba et al. 2010). Such induced mutations often produce genes or alleles not present in the natural population, increasing the chances of generating novel resistance traits (Novak and Brunner 1992). Genotypes showing desired traits could be used as parental genotypes for future breeding programs or further processed into varieties using systematic breeding procedures (Mba et al. 2010). Much focus, however, has been given to addressing pathogen resistance in crops using induced mutagenesis (Gottschalk and Wolff 2012; Oladosu et al. 2016) while few studies have considered using this approach to develop aphid resistant cultivars (Kharkwal et al. 2004; Gottschalk and Wolff 2012). Induced mutagenesis usually results 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 minor changes in amino acid composition (Mba et al. 2010). Since aphid resistance in crops is mediated by polygenic dominant alleles (Dogimont et al. 2010), creating dominant gain-of-gene function to produce novel aphicidal amino acids is rare using induced mutagenesis. However, owing to the lack of access to modern breeding tools particularly in SSA (Botha et al. 2020; Qaim 2020) as well as increasing legislative restrictions on insecticide use, induced mutagenesis could support genetic enhancement of cowpea for aphid resistance to mitigate the yield losses associated with these pests. Given the above mentioned potential of induced mutagenesis, it was hypothesised that gamma irradiation of cowpea genotypes would induce genetic variation for cowpea aphid resistance. This study identified aphid resistant genotypes after gamma

irradiation of three susceptible cowpea varieties widely grown in Zambia and the mechanism(s) underpinning this resistance.

MATERIALS AND METHODS

Plants

A total of eleven genotypes derived from three susceptible cowpea varieties were evaluated for aphid resistance. Susceptible cowpea varieties were Bubebe (BB), Lutembwe (LT) and Msandile (MS). Six genotypes BB 3-9-7-5 (BB3), BB 7-9-7-5 (BB7), BB 8-1-7-5 (BB8), BB 10-4-2-3 (BB10), BB 14-16-2-2 (BB14) and BBV (BBVN1) were derived from BB, three LT 3-8-4-6 (LT3), LT 4-2-4-1 (LT4) and LT 11-3-3-12 (LT11) from LT, and two MS 1-8-1-4 (MS1) and MS 10-7-2-1 (MS10) from MS. Seed from the susceptible varieties Bubebe and Lutembwe were treated with 150 gray of gamma rays while Msandile was treated at a lower dose of 100 gray because it was more sensitive to radiation. Radiation of seed was carried out using a Co60 source at the National Institute for Scientific and Industrial Research (NISIR), Plant Science Centre, Zambia. Resulting mutation derived genotypes were then advanced to stable generations 8 -10 (M_8 – M_{10}) before agronomic traits were evaluated. Genotypes used in this study were selected based on potential pesticidal traits including resistance to cowpea bruchid (*Callosobruchus maculatus* F.) (BB 7 and BB 14) (Tembo et al. 2017), anecdotal evidence of resistance to cowpea aphid (BB 10, BB 14 and LT 3) and cowpea leaf blight (*Ascochyta* spp.) (BB 8, LT 11 and LT 4).

Three seeds of each cowpea genotype were sown in plastic pots (diameter and height: 9 cm) (LBS worldwide Ltd., Lancashire, UK) containing potting soil (John Innes No. 2, J. Arthur Bower's, Westland Horticulture Limited, Cheshire, UK) and placed in an insect proof mesh cage (60 x 60 x 60 cm, BugDorm-6S610, MegaView Science Co. Ltd, Taichung, Taiwan) within a controlled environment room maintained at 20 °C and 60 % relative humidity with a 16:8 photoperiod (Fitotron, Weiss Technik UK limited, Loughborough, UK). Seeds were allowed to germinate and grow until they were eight days old (BBCH growth stage 10) (Lancashire et al. 1991) before being thinned to leave one seedling per pot. No fertiliser was applied to the plants and irrigation was done by adding water to trays twice weekly throughout the study period. Plants used for each of the bioassays completed were 10-15 days old (BBCH growth stage 11-15).

Aphid culture and age-synchronised cohort production

A stock culture of cowpea aphid (*Aphis craccivora* Koch) was reared on cowpea seedlings in an insect proof mesh cage (47.5 x 47.5 x 47.5 cm, BugDorm-4S4545, MegaView Science Co. Ltd, Taichung, Taiwan) within a controlled environment room maintained at 20 °C and 60 % relative humidity with a 16:8 photoperiod. The culture was maintained by transferring aphids onto new cowpea seedlings weekly throughout the study period.

To produce a cohort of age-synchronised apterous adult aphids for use in bioassays, two to five apterous adult aphids were transferred onto individual cowpea seedlings within an insect proof mesh cage. After 24 hours, adult aphids were removed from the plants using a size 000 paintbrush to leave only first instar nymphs. To prevent escape of nymphs and plants becoming infested with other insects, each plant was covered with a fine light-transmitting mesh bag (0.3 x 0.4 m large organza bags; mesh size 0.5 mm, TtS Ltd, UK), secured around the pot using an elastic band. Plants were maintained in a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod until the nymphs moulted into adults (approximately seven to eight days).

Cowpea aphid colony growth

Colony growth of cowpea aphid nymphs was assessed using a procedure adapted from Soffan and Aldawood (2014). Using a size 000 paintbrush, 1-2-day old age-synchronised adult apterous aphids were individually placed onto cowpea plant leaves in an insect proof mesh cage (as described before) within a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod. After 24 hours, all aphids were removed, leaving three first instar nymphs per plant. Each plant was covered with a fine light-transmitting mesh bag and returned to the controlled environment room. Fourteen days after infestation, total numbers of aphids (adults and nymphs) were counted and recorded. This experiment was replicated ten times for each cowpea genotype.

Individual cowpea aphid performance

Performance of individual cowpea aphids was evaluated as described by Hu et al. (2018). Aphids were individually placed onto cowpea plant leaves and maintained as described for the colony growth experiment. After 24 hours, all aphids were removed, leaving a single first instar nymph per plant. Each plant was covered with a fine light-transmitting mesh bag and returned to the controlled environment room. Nymphs were monitored daily to record development time, fecundity, intrinsic rate of natural increase (r_m), and population doubling time (DT). Measurement and calculation of each biological parameter was carried out as described in Table 1. A replicate was regarded as a single nymph placed on each cowpea genotype. This experiment was replicated fifteen times.

Mean relative growth rate of aphids was evaluated as described by Thieme and Heimbach, (1996). Aphids were individually placed onto cowpea plant leaves and maintained as described for the colony growth experiment. After 24 hours, all aphids were removed except ten first instar nymphs per plant which were weighed using a microbalance (XPR10 Ultra-microbalance, Mettler Toledo, Greifensee, Switzerland) to record the initial mean weight. After weighing, nymphs were placed back onto their respective plants and covered with a fine light-transmitting mesh bag. Plants were maintained in the controlled environment room for four days, when a single nymph from each plant was re-weighed to record the final weight. Mean relative growth rate was calculated as described in Table 1. Ten replications for each genotype were completed for the MRGR experiment.

Feeding behaviour

Direct-current (DC) electrical penetration graph (EPG) recording was used to monitor probing and feeding behaviour of apterous adult aphids (Tjallingii 1978). A plant probe, soldered to an electrical wire, was inserted into the moist soil of a potted plant while the free end of the wire was connected to the out-put voltage socket of the Giga-8-EPG device (EPG Systems, Wageningen, The Netherlands). An aphid probe was assembled by attaching a 3-4 cm piece of gold wire (diameter 20 µm, EPG Systems) to the copper electrode end of brass pin using conductive silver glue (EPG Systems). Using this glue, the other end of the gold wire was attached onto the aphid dorsum. Brass pins with wired aphids were then inserted into the EPG probes mounted on retort stands. The EPG probes were carefully lowered to allow aphids contact with leaves of wired individual plants. Feeding behaviour of eight aphids was monitored simultaneously over a four-hour period using a Giga-8-EPG device connected to a laptop computer. Twenty recordings were carried out for each cowpea genotype. Plants and aphids were contained in a grounded faraday cage during EPG recording.

Data was acquired using the stylet+ D software (EPG Systems) while waveforms; non-probing (np), pathway phase (pp), sieve element phase (SEP) and xylem ingestion (G) were annotated using the stylet+ A software (EPG Systems) based on the wave categories described by Tjallingii (1978). Annotated waveforms were transformed into time-series data using the Excel macro software developed by (Sarria et al. 2009).

Experimental design and data analysis

Due to the homogeneity of environmental conditions in the controlled environment room as well as soil used to grow plants, a complete randomised design (CRD) was used for all experiments. Statistical analyses were carried out using R version 4.0.2 (R Core Team 2020). Prior to analysis, key assumptions for parametric statistical tests were checked. Data distributions were checked using the Shapiro-Wilk test while homogeneity of variance was assessed by the Bartlett test. Data that satisfied parametric test assumptions were analysed using one-way analysis of variance (ANOVA). Non-Gaussian data that had non-homogenous variance were log-transformed to meet parametric assumptions before analysis using one-way ANOVA and pairwise comparisons with the Holm-Sidak method. Data that did not meet parametric assumptions following log-transformation such as for colony growth and feeding behaviour were analysed with Kruskal-Wallis rank-sum tests.

RESULTS

Cowpea aphid colony growth

The total number of aphids after fourteen days of colony development was influenced by plant genotype (Fig. 1). Significant differences in aphid colony growth were identified between the parent Bubebe and its associated genotypes derived through mutagenesis (Kruskal-Wallis: $X^2 = 19.67$, $df = 6$, $P < 0.01$) (Fig. 1A), with colony size reduced by 48.5 % on BB7, 31.6 % on BB14, 17.2 % on BB10 and, 5.8 % on BB3 (Fig. 1A). Similarly, significant

reductions in aphid colony growth were observed between mutation derived genotypes and the parent Lutembwe (Kruskal-Wallis: $X^2 = 18.16$, $df = 3$, $P < 0.001$) (Fig. 1B), with colony size reduced by 78.7 % on LT11, 69.3 % on LT3 and, 67.5 % on LT4 (Fig. 1B). Mutagenesis had no impact in reducing colony growth in genotypes derived from the parent Msandile (one-way ANOVA: $F = 2.19$, $df = 2$, $P > 0.05$) (Fig. 1C).

Performance of individual cowpea aphids

Aphid fecundity was significantly reduced on three genotypes (BB3, BB7 and BB10) derived from Bubebe through mutagenesis (one-way ANOVA: $F = 8.23$, $df = 6$, $P < 0.001$) (Table 2). Aphids reared on genotypes BB7, BB3 and BB10 had lower fecundity compared to the parent (Table 2). Aphid intrinsic rate of natural increase (r_m) differed between Bubebe and its derived genotypes (one-way ANOVA: $F = 4.44$, $df = 6$, $P < 0.001$) (Table 2). However, none of the genotypes reduced aphid r_m significantly when compared to the parent Bubebe (Table 2). Where aphid population doubling time was calculated, differences were identified between Bubebe and its associated genotypes derived through mutagenesis (one-way ANOVA: $F = 4.41$, $df = 6$, $P < 0.001$) (Table 2). None of the genotypes derived from Bubebe, however, increased aphid population doubling time compared to the parent (Table 2). Aphid mean relative growth rate (MRGR) differed between Bubebe and its derived genotypes produced through mutagenesis (one-way ANOVA: $F = 3.92$, $df = 6$, $P < 0.01$) (Table 2). However, none of the other genotypes affected aphid MRGR when compared to their parent (Bubebe) (Table 2). There were no differences in nymph development time between Bubebe and its derived genotypes (one-way ANOVA: $F = 0.80$, $df = 6$, $P > 0.05$) (Table 2).

Significant differences in aphid fecundity were observed between the parent Lutembwe and its derived genotypes LT3, LT4 and LT11 (one-way ANOVA: $F = 33.73$, $df = 3$, $P < 0.001$) (Table 3). Reduced fecundity was observed in aphids feeding on LT3, LT4 and LT11 compared to their parent (Table 3). Aphid r_m differed between Lutembwe and its derived genotypes (one-way ANOVA: $F = 11.75$, $df = 3$, $P < 0.001$) (Table 3). Genotypes LT3, LT4 and LT11 resulted in lower aphid r_m compared to their parent (Table 3). Where aphid population doubling time was calculated, differences were detected between Lutembwe and its derived genotypes (one-way ANOVA: $F = 10.76$, $df = 3$, $P < 0.001$) (Table 3). Genotypes LT3, LT4 and LT11 resulted in longer aphid population doubling time compared to the parent (Table 3). Differences in MRGR were identified between Lutembwe and its derived genotypes (one-way ANOVA: $F = 14.61$, $df = 3$, $P < 0.001$) (Table 3). On genotypes LT3, LT4 and LT11, recorded MRGRs were significantly lower than the parent (Table 3). No differences in nymph development time were detected between Lutembwe and derived genotypes (one-way ANOVA: $F = 1.60$, $df = 3$, $P > 0.05$) (Table 3).

Mutagenesis had no impact on promoting aphid resistant genotypes derived from the Msandile parent.

Feeding behaviour

Cowpea aphids showed differences in their feeding and probing behaviour within the leaf epidermis and mesophyll tissues (Figs. 2A and 2B). Significant differences in the duration of the first probe were observed between

the parent Lutembwe and genotypes derived from this parent through mutagenesis (one-way ANOVA: $F = 3.12$, $df = 3$, $P < 0.05$) (Fig. 2A). The first aphid probe on the parent was longer compared to the genotype LT11 (Fig. 2A). The pathway phase (duration until first phloem puncture) differed significantly between the parent and respective genotypes LT3, LT4 and LT11 (Kruskal-Wallis: $X^2 = 25.28$, $df = 3$, $P < 0.001$) (Fig. 2B). Pathway phase duration was longer on genotype LT4 compared to the parent (Fig. 2B). There were no differences between parent genotype and its derived genotypes in the duration of phloem salivation (Kruskal-Wallis: $X^2 = 7.96$, $df = 5$, $P > 0.05$) (Fig. 2C) or phloem ingestion (one-way ANOVA: $F = 1.07$, $df = 5$, $P > 0.05$) (Fig. 2D).

DISCUSSION

This study highlights the potential role that induced mutagenesis has in generating novel sources of resistance for breeding aphid resistant crop varieties. It is evident from this study that the population and biological parameters of the cowpea aphid were significantly influenced by mutation derived cowpea genotypes. A colony growth bioassay was initially conducted to screen cowpea genotypes for potential resistance traits. Colony growth on BB 7-9-7-5, LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 were lower when compared to their respective parents Bubebe and Lutembwe, indicating the presence of aphid resistance traits in these genotypes. Morphological (*e.g.*, trichomes) and biochemical (*e.g.*, alkaloids, phenols, flavonoids) traits are known to influence cowpea aphid performance on cowpea (Ofuya 1997). Lower aphid colony growth on BB 7-9-7-5, LT 3-8-4-1, LT 4-2-4-2 and LT 11-3-3-12 reflects reduced host quality of these genotypes (Soffan and Aldawood 2014), likely due to mutagenesis derived resistance traits (Viana et al. 2019). Results obtained from the colony development bioassay, however, need to be tested under field conditions to establish whether they can be replicated outside of the laboratory.

Although nymph development was not found to be lower on any cowpea genotypes produced through mutagenesis, there was a general trend of extended nymph development on BB 3-9-7-5, BB 8-1-7-5, BB 10-4-2-3 and LT 11-3-3-12 compared to their respective parents. Extended nymph development on these genotypes may indicate host resistance since aphid resistant traits are often associated with delayed adult emergence (Zimba, Sohati, Munyinda, Kamfwa, et al. 2022). However, it will be important to screen the promising genotypes with a range of widespread aphid clones to establish if the results reported here are consistent for a wider range of aphid biotypes. Particularly, such studies would help to assess if the promising genotypes in this study are effective against the previously reported resistance breaking aphid biotypes.

Cowpea aphid fecundity was reduced on genotypes BB 7-9-7-5, BB 3-9-7-5, BB 10-4-2-3, LT 3-8-4-1, LT 4-2-4-2 and LT 11-3-3-12 compared to their respective parents, possibly indicating the presence of resistance factors including reduced nutrition quality of these genotypes. Genetic effects of induced mutagenesis such as base substitution and gene deletion (Viana et al. 2019) may have led to changes in the composition of amino acids and secondary metabolites (*i.e.*, polyphenols and flavonoids) in these genotypes, which could have led to poor nutrition and therefore lower fecundity of aphids. Indeed, Douglas and Prosser (1992) showed that exclusion of essential amino acids such as tryptophan in artificial diets reduced the fitness of the pea aphid (*Acyrthosiphon pisum*). Lattanzio et al.

(2000) also demonstrated that high levels of flavonoids, such as quercetin and isorhamnetin, in cowpea genotypes inhibited cowpea aphid reproduction. Mean relative growth rate (MRGR) is often used as a predictor of aphid reproductive performance since lower weight gains are correlated with reduced fecundity (Obopile and Ositile 2010). Lower aphid MRGRs on LT 3-8-4-1, LT 4-2-4-2, and LT 11-3-3-12 were also associated with lower fecundity compared to the parent, which may suggest reduced food quality of these genotypes due to potential resistant factors described above. Phytochemicals and low nutritional values associated with resistant crop cultivars may reduce fitness of omnivorous natural enemies of aphids (Lundgren et al. 2008). Therefore further studies are needed to assess if promising aphid resistant genotypes are compatible with natural enemies within IPM systems (Michereff et al. 2015).

Intrinsic rate of natural increase (r_m) is a function of nymph development (d) and fecundity (M_d) (Wyatt and White 1977). This development metric is a useful summary parameter that provides an estimate of aphid performance when reared on different host plants and has been widely used to evaluate aphid resistance in crop cultivars (Obopile and Ositile 2010, Leybourne et al. 2019). Higher r_m values indicate greater growth potential when aphid populations are reared on susceptible host plants (Dixon 1998). Genotypes LT 3-8-4-1, LT 4-2-4-2 and LT 11-3-3-12 resulted in lower aphid r_m compared to their parent, indicating host plant resistance and an inhibition of aphid population growth. This corroborates with findings by Obopile and Ositile (2010) and Soffan and Aldawood (2014) who reported significantly lower values of aphid r_m when reared on resistant genotypes of cowpea and broad bean (*Vicia faba* L.) respectively. Indeed, aphid colony growth on genotypes LT 3-8-4-1, LT 4-2-4-2 and LT 11-3-3-12 was reduced suggesting poor aphid performance on these lines.

Population doubling time (DT) is the time it takes for the aphid population to double in size. Aphid populations took, on average, 0.3 days longer to double on genotypes LT 3-8-4-1, LT 4-2-4-2 and LT 11-3-3-12 compared to their parent genotype. Feeding analysis by EPG indicated a reduced first probe (duration of first stylet movement within the leaf epidermal layer) duration on LT 11-3-3-12, suggesting the presence of epidermal barriers (*i.e.*, epicuticular chemical compounds) to leaf penetration by the aphid stylet (Leybourne et al. 2019). Moreover, the longer pathway phase (duration of stylet movement from leaf surface until phloem puncture) in these genotypes may further suggest the presence of resistance factors in the mesophyll. Previous studies have demonstrated the contribution of epidermal (Leybourne et al. 2019) and mesophyll (Kamphuis et al. 2012) based aphid resistance factors in plants. However, biochemical and morphological characterisation of cowpea leaves would be useful in future studies.

Genotypes BB 7-9-7-5 and BB 14-16-2-2 are resistant to cowpea bruchid (*C. maculatus*) (Tembo et al. 2017). Although BB 14-16-2-2 did not affect colony growth or most indicators of individual cowpea aphid performance, reduced aphid fecundity observed on BB 7-9-7-5 suggests cross-resistance to cowpea aphid. BB 7-9-7-5 resistance to cowpea aphid and cowpea bruchid may be mediated by biochemical compounds that have broader insecticidal activity, such as alpha-amylase inhibitors, tannins, phenolic compounds, lectins and protease inhibitors (War et al. 2012). Previous field observations on genotypes BB 8-1-7-5, LT 11-3-3-12 and LT 4-2-4-2 indicated low incidences of the leaf blight (*Ascochyta* spp.) (unpublished). While BB 8-1-7-5 did not affect cowpea aphid biology, several parameters (nymph development, fecundity, MRGR, r_m and DT) were adversely affected by genotypes LT 11-3-3-12 and LT 4-

2-4-2, which may further indicate resistance to both leaf blight and cowpea aphid. Aphid and pathogen resistance genes are often clustered on the same region of the chromosomes (Dogimont et al. 2010). For example, the *Ra* gene on chromosome 2 in lettuce, which mediates resistance against the lettuce root aphid (*Pemphigus bursarius* L.), is clustered together with downy mildew resistance genes on the chromosome (Wroblewski et al. 2007; Christopoulou et al. 2015).Typically, plants respond to aphid feeding in a similar way to plant pathogens (Zimba, Sohati, Munyinda, Roberts, et al. 2022). Due to this common genomic locale of aphid and pathogen resistance genes, supposed chromosomal alterations due to mutagenesis in LT 4-2-4-2 and LT 11-3-3-12 may have induced genetic variations for both pathogen and aphid resistance traits. However, genetic characterisation of genotypes BB 7, LT 4-2-4-2 and LT 11-3-3-12 requires further work to elucidate mechanisms of resistance.

In conclusion, this study shows that aphids reared on genotypes LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12 that were produced through mutagenesis had lower colony growth, fecundity, MRGR, r_m and DT compared to the parent. Among the genotypes derived from the parent Bubebe through mutagenesis, genotype BB7 had the effect of significantly reducing cowpea aphid colony growth compared to the parent. Characterisation of aphid probing and feeding behaviour using EPG indicates that resistance factors in genotypes LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12 may predominantly reside within the epidermal and mesophyll tissues of cowpea leaves. Genotypes BB 7-9-7-5, LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12 are therefore promising lines that should be further evaluated for useful genetic attributes that may be used to develop aphid resistant cowpea varieties. Although developing aphid resistance using induced mutagenesis is associated with several challenges as highlighted above, this study shows that using this approach could contribute to sustainable management of aphid pests in crops. 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 SSA.

ACKNOWLEDGEMENTS

This study was supported by funds from the International Atomic Energy Agency (IAEA) through a sandwich PhD fellowship awarded to the first author (Grant number: RAF0052-1805114). Provision of cowpea seed used in this study from the Department of Plant Science at the University of Zambia is greatly acknowledged.

AUTHOR CONTRIBUTIONS

KJZ, TWP and JMR conceived and designed the paper. KJZ wrote the manuscript while TWP, JMR, PHS and KM critically reviewed and edited the draft. All authors approved the final manuscript.

Availability of data and material

Data that support the findings of this study are available from the corresponding author (KJZ), upon reasonable request.

Code availability

Not available.

DECLARATIONS

Conflict of interest

Authors have no conflict of interest to declare.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Agbicodo, E. M., C. A. Fatokun, S. Muranaka, R. G. F. Visser, and C. G. Linden Van Der. **2009**. Breeding drought tolerant cowpea: Constraints, accomplishments, and future prospects. Euphytica. 167: 353–370.

Atiri, G. I., D. A. Enobakhare, and G. Thottappilly. **1986**. The importance of colonizing and non-colonizing aphid vectors in the spread of cowpea aphid-borne mosaic virus in cowpea. Crop Prot. 5: 406–410.

Bata, H. D., B. B. Singh, S. R. Singh, and T. A. O. Ladeinde. **1987**. Inheritance of resistance to aphid in cowpea. Crop Sci. 27: 892–894.

Botha, A. M., K. J. Kunert, J. Maling'a, and C. H. Foyer. **2020**. Defining biotechnological solutions for insect

control in sub-Saharan Africa. Food Energy Secur. 9: 1–21.

Boukar, O., N. Belko, S. Chamarthi, A. Togola, J. Batieno, E. Owusu, M. Haruna, S. Diallo, M. L. Umar, O. Olufajo, and C. Fatokun. **2019**. Cowpea (Vigna unguiculata): Genetics, genomics and breeding. Plant Breed. 138: 415–424.

Chen, M. H., Z. J. Han, X. F. Qiao, and M. J. Qu. **2007**. Mutations in acetylcholinesterase genes of Rhopalosiphum padi resistant to organophosphate and carbamate insecticides. Genome. 50: 172–179.

Chougule, N. P., and B. C. Bonning. **2012**. Toxins for transgenic resistance to hemipteran pests. Toxins (Basel). 4: 405–429.

Christopoulou, M., L. K. McHale, A. Kozik, S. R. C. Wo, T. Wroblewski, and R. W. Michelmore. **2015**. Dissection of two complex clusters of resistance genes in lettuce (Lactuca sativa). Mol. Plant-Microbe Interact. 28: 751–765.

Desneux, N., A. Decourtye, and J.-M. Delpuech. **2006**. The Sublethal Effects of Pesticides on Beneficial Arthropods. Annu. Rev. Entomol. 52: 81–106.

Dixon, A. F. G. 1998. Aphid Ecology: an Optimization Approach, 2nd edition. Chapman and Hall, London, UK, Second Edi. ed. London.

Dogimont, C., A. Bendahmane, V. Chovelon, and N. Boissot. **2010**. Host plant resistance to aphids in cultivated crops: Genetic and molecular bases, and interactions with aphid populations. Comptes Rendus - Biol. 333: 566–573.

Douglas, A. E., and W. A. Prosser. **1992**. Synthesis of the essential amino acid tryptophan in the pea aphid (Acyrthosiphon pisum) symbiosis. J. Insect Physiol. 38: 565–568.

Ehlers, J. D., and A. E. Hall. **1996**. Genotypic classification of cowpea based on responses to heat and photoperiod. Crop Sci. 36: 673–679.

Elowad, H. O., and A. E. Hall. **1987**. Influences of early and late nitrogen fertilization on yield and nitrogen fixation of cowpea under well-watered and dry field conditions. F. Crop. Res. 15: 229–244.

Ezeaku, I. E., B. N. Mbah, and K. P. Baiyeri. **2017**. Response of cowpea (Vigna unguiculata (l.) walp) genotypes to sowing dates and insecticide spray in South Eastern Nigeria. J. Anim. Plant Sci. 27: 239–245.

Foster, S. P., V. L. Paul, R. Slater, A. Warren, I. Denholm, L. M. Field, and M. S. Williamson. **2014**. A mutation (L1014F) in the voltage-gated sodium channel of the grain aphid, Sitobion avenae, is associated with resistance to pyrethroid insecticides. Pest Manag. Sci. 70: 1249–1253.

Gottschalk, W., and G. Wolff. **2012**. Induced mutations in plant breeding (Vol. 7).

Holland, J., B. Bown, J. Clarke, and N. Mchugh. **2019**. Patterns of cereal aphid infestation in autumn and

implications for Barley Yellow Dwarf Virus control. 143: 105–109.

Horn, L. N., H. M. Ghebrehiwot, and H. A. Shimelis. **2016**. Selection of novel cowpea genotypes derived through gamma irradiation. Front. Plant Sci. 7: 1–13.

Hu, X. S., Z. F. Zhang, T. Y. Zhu, Y. Song, L. J. Wu, X. F. Liu, H. Y. Zhao, and T. X. Liu. **2018**. Maternal effects of the English grain aphids feeding on the wheat varieties with different resistance traits. Sci. Rep. 8: 1–11.

Huynh, B. L., J. D. Ehlers, A. Ndeve, S. Wanamaker, M. R. Lucas, T. J. Close, and P. A. Roberts. **2015**. Genetic mapping and legume synteny of aphid resistance in African cowpea (Vigna unguiculata L. Walp.) grown in California. Mol. Breed. 35.

Jackai, L. E. N., and R. A. Daoust. **1986**. Insect pests of cowpeas. Annu. Rev. Entomol. 31: 95–119.

James, K. L., N. P. Randall, K. F. A. Walters, N. R. Haddaway, and M. Land. **2016**. Evidence for the effects of neonicotinoids used in arable crop production on non-target organisms and concentrations of residues in relevant matrices: a systematic map protocol. Environ. Evid. 5: 1–9.

Jaouannet, M., P. A. Rodriguez, P. Thorpe, C. J. G. Lenoir, R. Macleod, C. Escudero-Martinez, and J. I. B. Bos. **2014**. Plant immunity in plant-aphid interactions. Front. Plant Sci. 5: 1–10.

Jayathilake, C., R. Visvanathan, A. Deen, R. Bangamuwage, B. C. Jayawardana, S. Nammi, and R. Liyanage. **2018**. Cowpea: an overview on its nutritional facts and health benefits. J. Sci. Food Agric. 98: 4793–4806.

Kamphuis, L. G., L. Gao, and K. B. Singh. **2012**. Identification and characterization of resistance to cowpea aphid (Aphis craccivora Koch) in Medicago truncatula.

Kharkwal, M. C., R. N. Pandey, and S. E. Pawar. **2004**. Mutation breeding for crop improvement, pp. 610–612. *In* Plant Breed. springer Dordrecht.

Lancashire, P. D., H. Bleiholder, T. V. D. Boom, P. Langelüddeke, R. Stauss, E. Weber, and A. Witzenberger. **1991**. A uniform decimal code for growth stages of crops and weeds. Ann. Appl. Biol. 119: 561–601.

Lattanzio, V., S. Arpaia, A. Cardinali, and D. Di Venere. **2000**. Role of Endogenous Flavonoids in Resistance Mechanism of Vigna to Aphids Role of Endogenous Flavonoids in Resistance Mechanism of Vigna.

Leybourne, D. J., T. A. Valentine, J. A. H. Robertson, E. Pérez-Fernández, A. M. Main, A. J. Karley, and J. I. B. Bos. **2019**. Defence gene expression and phloem quality contribute to mesophyll and phloem resistance to aphids in wild barley. J. Exp. Bot. 70: 4011–4026.

Lundgren, J. G., J. K. Fergen, and W. E. Riedell. **2008**. The influence of plant anatomy on oviposition and reproductive success of the omnivorous bug Orius insidiosus. Anim. Behav. 75: 1495–1502.

Mba, C., R. Afza, S. Bado, and S. M. Jain. **2010**. Induced mutagenesis in plants using physical and chemical

agents, pp. 111–130. *In* Plant Cell Cult. Essent. Methods.

Michereff, M. F. F., M. Michereff Filho, M. C. Blassioli-Moraes, R. A. Laumann, I. R. Diniz, and M. Borges. **2015**. Effect of resistant and susceptible soybean cultivars on the attraction of egg parasitoids under field conditions. J. Appl. Entomol. 139: 207–216.

Musenga, C., D. Mwaba, V. Kilubi, K. Bwalya, J. Mweembe, B. Siame, M. Mtawa, R. Kelly, A. K. Chipuluka, C. Chilala, D. M. Ndalamei, K. Muchula, and I. Ntenga. **2016**. Pest Management Decision Guide: Green and Yellow List, Cowpea aphids on cowpeas. Zambia Agricultural Research Institute (ZARI)-Plantwise. CABI. (https://www.plantwise.org/KnowledgeBank/pmdg/20177800703).

Nalam, V. J., J. Louis, and J. Shah. **2019**. Plant defense against aphids, the pest extraordinaire. Plant Sci. 279: 96–107.

Novak, F. J., and H. Brunner. **1992**. Plant breeding: Induced mutation technology for crop improvement. IAEA Bull. 4: 24–33.

Obopile, M., and B. Ositile. **2010**. Life table and population parameters of cowpea aphid , Aphis craccivora Koch (Homoptera : Aphididae) on five cowpea Vigna unguiculata (L . Walp .) varieties. 9–14.

Ofuya, T. I. 1997. Control of the cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae), in cowpea, *Vigna unguiculata* (L.) Walp. Integr. Pest Manag. Rev. 2: 199–207.

Okoth, J. K., S. A. Ochola, N. K. Gikonyo, and A. Makokha. **2017**. Development of a nutrient-dense complementary food using amaranth-sorghum grains. Food Sci. Nutr. 5: 86–93.

Oladosu, Y., M. Y. Rafii, N. Abdullah, G. Hussin, A. Ramli, H. A. Rahim, G. Miah, and M. Usman. **2016**. Principle and application of plant mutagenesis in crop improvement: A review. Biotechnol. Biotechnol. Equip. 30: 1–16.

Olasupo, F. O., C. O. Ilori, B. P. Forster, and S. Bado. **2018**. Selection for Novel Mutations Induced by Gamma Irradiation in Cowpea [Vigna unguiculata (L.) Walp.]. Int. J. Plant Breed. Genet. 12: 1–12.

Ombakho, G. A., A. P. Tyagi, and R. S. Pathak. **1987**. Inheritance of resistance to the cowpea aphid in cowpea. Theor. Appl. Genet. 74: 817–819.

Ouédraogo, A. P., B. J. Batieno, F. Traore, J. Tignegre, L. Huynh, P. A. Roberts, T. Close, and J. T. Ouédraogo. **2018**. Screening of cowpea (Vigna unguiculata (L .) Walp .) lines for resistance to three Aphids (Aphis craccivora Koch) strains in Burkina Faso. 13: 1487–1495.

Pertot, I., T. Caffi, V. Rossi, L. Mugnai, C. Hoffmann, M. S. Grando, C. Gary, D. Lafond, C. Duso, D. Thiery, V. Mazzoni, and G. Anfora. **2017**. A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. Crop Prot. 97: 70–84.

Pettersson, J., S. Karunaratne, E. Ahmed, and V. Kumar. **1998**. The cowpea aphid , Aphis craccivora , host plant odours and pheromones. 177–184.

Qaim, M. 2020. Role of New Plant Breeding Technologies for Food Security and Sustainable Agricultural Development. Appl. Econ. Perspect. Policy. 42: 129–150.

R Core Team. **2020**. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reddy, S. G. E., S. K. Dolma, P. K. Verma, and B. Singh. **2018**. Insecticidal activities of Parthenium hysterophorus L. extract and parthenin against diamondback moth, Plutella xylostella (L.) and aphid, Aphis craccivora Koch. Toxin Rev. 37: 161–165.

Samireddypalle, A., O. Boukar, E. Grings, C. A. Fatokun, P. Kodukula, R. Devulapalli, I. Okike, and M. Blümmel. **2017**. Cowpea and groundnut haulms fodder trading and its lessons for multidimensional cowpea improvement for mixed crop livestock systems in west Africa. Front. Plant Sci. 8: 1–9.

Saranya, S., R. Ushakumari, S. Jacob, and B. M. Philip. **2010**. Efficacy of different entomopathogenic fungi against cowpea aphid, Aphis craccivora (Koch). J. Biopestic. 3: 138–142.

Sarria, E., M. Cid, E. Garzo, and A. Fereres. **2009**. Workbook for automatic parameter calculation of EPG data. Comput. Electron. Agric. 67: 35–42.

Sikora, P., A. Chawade, M. Larsson, J. Olsson, and O. Olsson. **2011**. Mutagenesis as a tool in plant genetics, functional genomics, and breeding. Int. J. Plant Genomics. 2011.

Smith, C. M., and W. Chuang. **2014**. Plant resistance to aphid feeding : behavioral , physiological , genetic and molecular cues regulate aphid host selection and feeding. Pest Manag. Sci. 70: 528–540.

Soffan, A., and A. S. Aldawood. **2014**. Biology and Demographic Growth Parameters of Cowpea Aphid (Aphis craccivora) on Faba Bean (Vicia faba) Cultivars. J. Insect Sci. 14: 1–10.

Stout, M., and J. Davis. **2009**. Keys to the increased use of host plant resistance in integrated pest management, pp. 163–181. *In* Integr. Pest Manag. Innov. Process. springer Dordrecht Heidelberg.

Taiwo, M. A., K. T. Kareem, I. Y. Nsa, and J. D'A Hughes. **2007**. Cowpea viruses: Effect of single and mixed infections on symptomatology and virus concentration. Virol. J. 4: 1–5.

Tembo, L., L. Pungulani, P. Sohati, J. Mataa, and M. Kalaluka. **2017**. Journal of Agriculture and Crops Resistance to Callosobruchus maculatus Developed Via Gamma Radiation in Cowpea. 3: 65–71.

Thieme, T., and U. Heimbach. **1996**. Development and reproductive potential of cereal aphids (Homoptera: Aphididae) on winter wheat cultivars. Bull. OILB SROP. 19: 1–8.

Tjallingii, W. F. 1978. Electronic recording of penetration behaviour by aphids. Entomol. Exp. Appl. 24: 721–730.

Viana, V. E., C. Pegoraro, C. Busanello, and A. Costa de Oliveira. **2019**. Mutagenesis in Rice: The Basis for Breeding a New Super Plant. Front. Plant Sci. 10: 1–28.

War, A. R., M. G. Paulraj, T. Ahmad, A. A. Buhroo, B. Hussain, S. Ignacimuthu, and H. C. Sharma. **2012**. Mechanisms of plant defense against insect herbivores. Plant Signal. Behav. 7.

Wilkinson, T. L., and A. E. Douglas. **2003**. Phloem amino acids and the host plant range of the polyphagous aphid, Aphis fabae. Entomol. Exp. Appl.

Wroblewski, T., U. Piskurewicz, A. Tomczak, O. Ochoa, and R. W. Michelmore. **2007**. Silencing of the major family of NBS-LRR-encoding genes in lettuce results in the loss of multiple resistance specificities. Plant J. 51: 803–818.

Wyatt, I. J., and P. F. White. **1977**. Simple estimation of intrinsic increase rates for aphids and tetranychid mites. J. Appl. Ecol. 14: 757–766.

Yates, A. D., and A. Michel. **2018**. Mechanisms of aphid adaptation to host plant resistance. Curr. Opin. Insect Sci. 26: 41–49.

Zhu-Salzman, K., and R. Zeng. **2015**. Insect Response to Plant Defensive Protease Inhibitors. Annu. Rev. Entomol. 60: 233–252.

Zimba, K. J., P. H. Sohati, K. Munyinda, K. Kamfwa, J. M. Roberts, and T. W. Pope. **2022**. Evaluation of resistance to black bean aphid (Aphis fabae) in selected varieties and mutant genotypes of common bean (Phaseolus vulgaris). Ann. Appl. Biol.

Zimba, K. J., P. H. Sohati, K. Munyinda, J. M. Roberts, and T. W. Pope. **2022**. Induced mutagenesis: An underutilised component in the integrated management of aphid pests in sub-Saharan Africa. Crop Prot. 159: 106030.

Züst, T., and A. A. Agrawal. **2016**. Mechanisms and evolution of plant resistance to aphids. Nat. Plants. 2: 1–9.

TABLES

TABLE 1 Description of aphid parameters measured in the study

Aphid parameter	**measurement**
Nymph development (*d*)	Duration from birth to onset of reproduction
Fecundity (M_d)	Total number of nymphs born from an individual aphid after adult emergence within a duration equivalent to *d*
Intrinsic rate of natural increase (r_m)	$\frac{0.738 \text{ In } (Md)}{d}$
Population doubling time (DT)	$\frac{\text{In } (2)}{rm}$
Mean relative growth rate (MRGR)	$\frac{(\log(W_2) - \log(W_1)}{(t_2 - t_1)}$

W_1 = initial mean weight of nymphs, W_2 = weight of a single nymph after four days, ($t_2 - t_1$) = period (days) between the initial (t_1) and final weighing (t_2).

TABLE 2 Performance of cowpea aphid on Bubebe genotypes (mean ± SE). SE = standard error. n = 10. Means followed by the same letter in the same row are not significantly different (Holm-Sidak post-hoc test). d = nymph development, M_d = fecundity, r_m = intrinsic rate of natural increase, DT = development time and, MRGR = mean relative growth rate

Parameters	Cowpea genotypes						
	Bubebe	**BB 3-9-7-5**	**BB 7-9-7-5**	**BB 8-1-7-5**	**BB 10-4-2-3**	**BB 14-16-2-2**	**BB VN1**
d	7.73 ± 0.118 a	7.87 ± 0.192 a	7.73 ± 0.153 a	7.60 ± 0.131 a	7.67 ± 0.126 a	7.60 ± 0.131 a	7.47 ± 0.133 a
M_d	62.00 ± 3.925 c	42.67 ± 2.499 a	42.53 ± 1.518 a	60.73 ± 2.726 bc	48.53 ± 3.458 ab	51.27 ± 3.078 abc	62.00 ± 2.926 c
r_m	0.39 ± 0.006 abc	0.36 ± 0.010 ab	0.36 ± 0.009 a	0.40 ± 0.010 bc	0.37 ± 0.010 abc	0.38 ± 0.009 abc	0.41 ± 0.009 c
DT	1.78 ± 0.031 ab	1.94 ± 0.051 b	1.94 ± 0.046 b	1.75 ± 0.043 ab	1.89 ± 0.052 ab	1.83 ± 0.040 ab	1.71 ± 0.040 a
MRGR	0.16 ± 0.013 ab	0.14 ± 0.010 a	0.13 ± 0.014 a	0.15 ± 0.005 b	0.14 ± 0.009 a	0.19 ± 0.009 ab	0.18 ± 0.016 ab

TABLE 3 Performance of cowpea aphid on Lutembwe genotypes (mean ± SE). SE = standard error. n = 10. Means followed by the same letter in the same row are not significantly different (Holm-Sidak post-hoc test). d = nymph development, M_d = fecundity, r_m = intrinsic rate of natural increase, DT = development time and, MRGR = mean relative growth rate

Parameters	Cowpea genotypes			
	Lutembwe	**LT 3-8-4-6**	**LT 4-2-4-1**	**LT 11-3-3-12**
d	7.53 ± 0.133 a	7.40 ± 0.131 a	7.53 ± 0.215 a	7.87 ± 0.133 a
M_d	65.60 ± 1.740 b	39.33 ± 2.464 a	42.73 ± 2.357 a	37.00 ± 2.430 a
r_m	0.41 ± 0.008 b	0.36 ± 0.007 a	0.37 ± 0.010 a	0.34 ± 0.010 a
DT	1.70 ± 0.032 b	1.91 ± 0.036 a	1.90 ± 0.059 a	2.08 ± 0.057 a
MRGR	0.18 ± 0.009 b	0.11 ± 0.012 a	0.10 ± 0.007 a	0.09 ± 0.015 a

TABLE 4 Performance of cowpea aphid on Msandile genotypes (mean ± SE). SE = standard error. n = 10. Means followed by the same letter in the same row are not significantly different (Holm-Sidak post-hoc test). d = nymph development, M_d = fecundity, r_m = intrinsic rate of natural increase, DT = development time and, MRGR = mean relative growth rate

Parameters	**Cowpea genotypes**		
	Msandile	**MS 1-8-1-4**	**MS 10-11-1-1**
d	7.60 ± 0.163	7.40 ± 0.131	7.60 ± 0.163
M_d	72.33 ± 2.425	72.87 ± 2.569	75.67 ± 2.259
r_m	0.42 ± 0.007	0.43 ± 0.005	0.42 ± 0.008
DT	1.67 ± 0.031	1.62 ± 0.020	1.65 ± 0.034
MRGR	0.20 ± 0.011	0.17 ± 0.013	0.20 ± 0.010

FIGURE LEGEND

Fig. 1 Aphid colony growth on (A) Bubebe, (B) Lutembwe and, (C) Msandile genotypes. *n* = 10. Error bars represent standard error of the mean (SE), Bars followed by different letters are significantly different (Holm-Sidak post-hoc test), ns = non-significant differences among bars.

Fig. 2 Aphid probing and feeding behaviour on cowpea genotypes. (A) duration of first aphid probe, (B) duration until first phloem puncture, (C) total duration of phloem salivation and, (D) total duration of phloem ingestion. (E) total time of phloem salivation. Error bars represent standard error of the mean (SE), Bars followed by different letters are significantly different (Holm-Sidak post-hoc test), ns = non-significant differences among bars. *n* = 12.

FIGURES

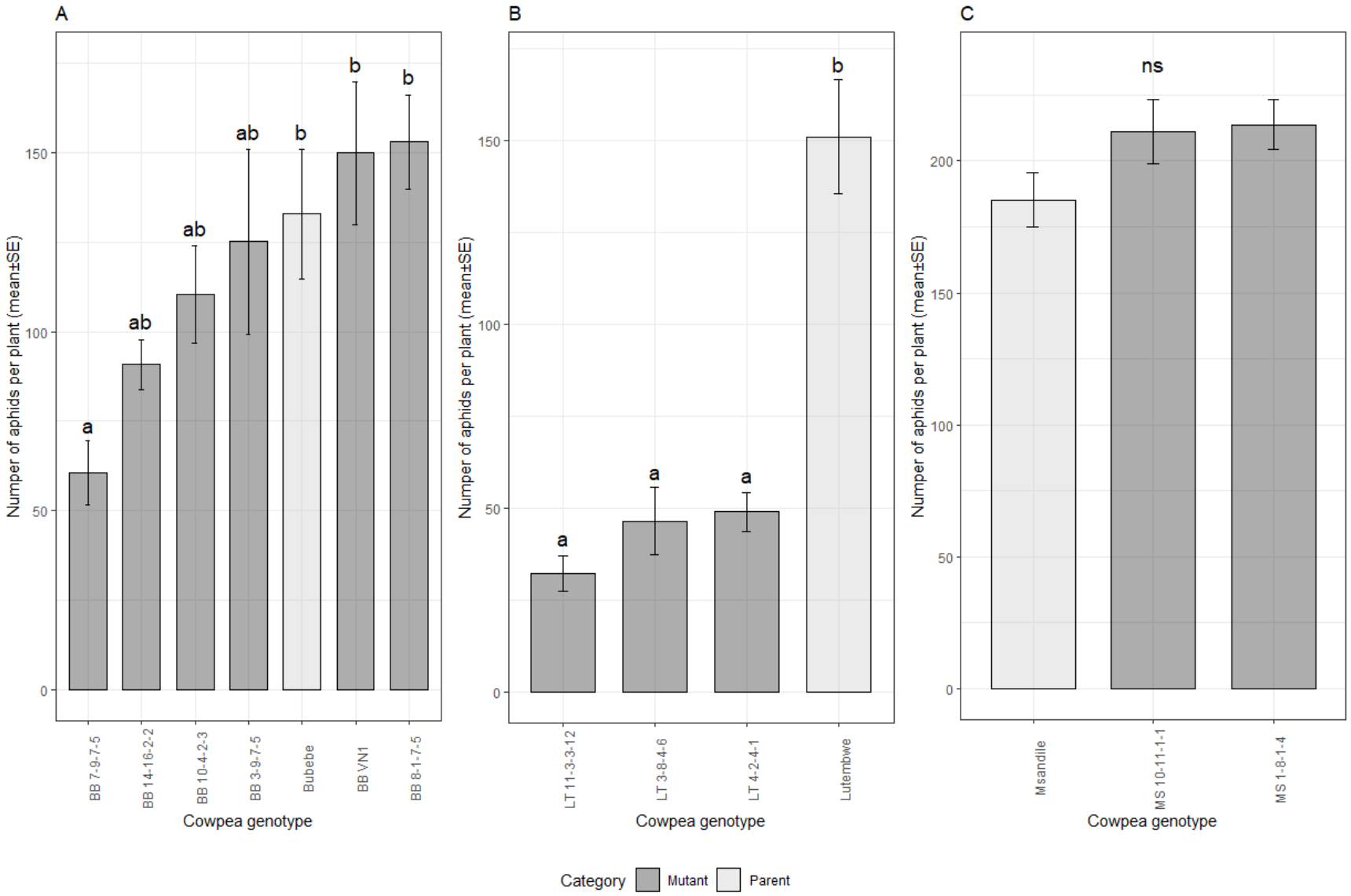
A
B
C
Numper of aphids per plant (mean±SE)
Cowpea genotype
a
ab
ab
ab
b
b
a
a
a
b
ns
BB 7-9-7-5
BB 14-16-2-2
BB 10-4-2-3
BB 3-9-7-5
Bubebe
BB VN1
BB 8-1-7-5
LT 11-3-3-12
LT 3-8-4-6
LT 4-2-4-1
Lutembwe
Msandile
MS 10-11-1-1
MS 1-8-1-4
Category
Mutant
Parent

Fig. 1 Aphid colony growth on (A) Bubebe, (B) Lutembwe and, (C) Msandile genotypes. *n* = 10. Error bars represent standard error of the mean (SE), Bars followed by different letters are significantly different (Holm-Sidak post-hoc test), ns = non-significant differences among bars

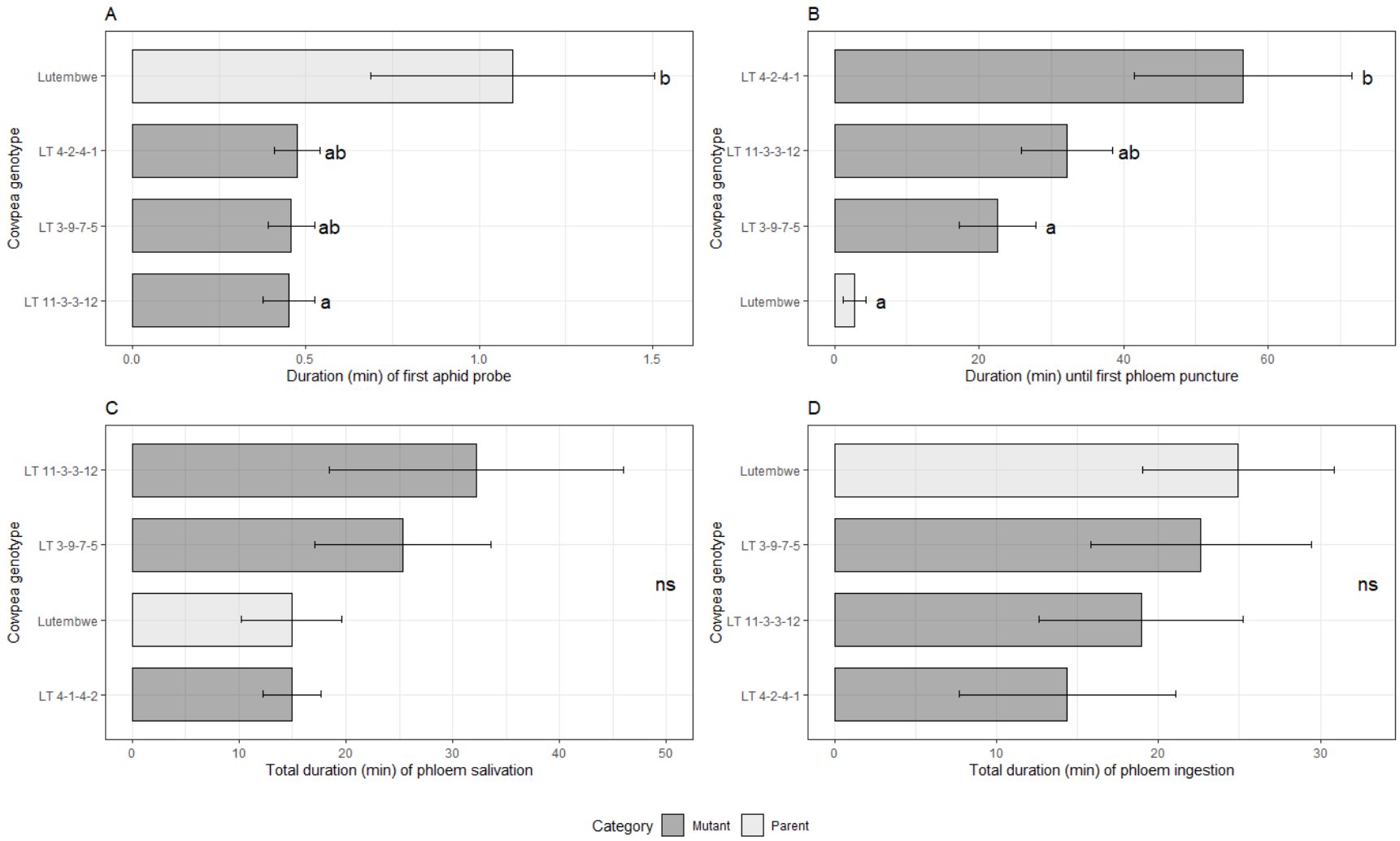

Fig. 2 Aphid probing and feeding behaviour on cowpea genotypes. (A) duration of first aphid probe, (B) duration until first phloem puncture, (C) total duration of phloem salivation and, (D) total duration of phloem ingestion. (E) total time of phloem salivation. Error bars represent standard error of the mean (SE), Bars followed by different letters are significantly different (Holm-Sidak post-hoc test), ns = non-significant differences among bars. $n = 12$.