Evaluation of resistance to black bean aphid (Aphis fabae) in selected varieties and mutant genotypes of common bean (Phaseolus vulgaris)

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

Copyright, publisher and additional information: This is the authors' accepted manuscript. The published version is available via Wiley.

Please refer to any applicable terms of use of the publisher

DOI link to the version of record on the publisher's site

Harper Adams University

Zimba, K.J., Sohati, P.H., Munyinda, K., Kamfwa, K., Roberts, J.M. and Pope, T.W. (2022) 'Evaluation of resistance to black bean aphid (Aphis fabae) in selected varieties and mutant genotypes of common bean (Phaseolus vulgaris)', *Annals of Applied Biology*

12 May 2022

1 **Running head:** Resistance to aphid in common bean genotypes

2

3 **Evaluation of resistance to black bean aphid (*Aphis fabae*) in selected varieties and mutant**
4 **genotypes of common bean (*Phaseolus vulgaris*)**

5

6 **Kennedy J. Zimba [1,2] | Philemon H. Sohati [2] | Kalaluka Munyinda [2] | Kelvin Kamfwa [2] | Joe M.**
7 **Roberts[1] | Tom W. Pope[1]**

8

9 [1] Centre for Integrated Pest Management, Agriculture and Environment Department, Harper
10 Adams University, Newport, Shropshire, TF10 8NB, UK,

11 [2] School of Agricultural Sciences, Department of Plant Sciences, University of Zambia, Great East
12 Road Campus, Lusaka 10101, Zambia.

13

14 **Correspondence**

15 Kennedy J. Zimba, Centre for Integrated Pest Management, Agriculture and Environment
16 Department, Harper Adams University, Newport, Shropshire, TF10 8NB, UK. Email:
17 zimbakj@gmail.com

18

19

20

21

22

23

24

25

26

27

28

1 **Abstract**

2 Common bean (*Phaseolus vulgaris*) is an important food crop across sub-Saharan Africa.
3 In Zambia, actual common bean yields are typically lower than potential yields due to the impact
4 of invertebrate pests and plant diseases. Black bean aphids (*Aphis fabae*) (Hemiptera: Aphididae)
5 negatively impact bean productivity directly by ingesting plant assimilates and indirectly by
6 vectoring diseases such as bean common mosaic virus (BCMV). Current breeding programs aim
7 to develop bean cultivars with improved yield and tolerance to pests. The objective of this study
8 was to screen five common bean varieties (Rozi Koko, Mwezi Moja, Majesty, KK25 and AO-1012-
9 29-3A) and four mutation-derived genotypes (CA 3, CA 15, CA 24 and CA 38) for resistance to
10 black bean aphid. Commercial bean cultivars, Kabulangeti and Carioca (variety from which all
11 mutants were derived) were used as controls for the selected varieties and mutant genotypes,
12 respectively. Several parameters of aphid resistance traits were assessed. Deterrence to aphid
13 was assessed by settling preference while physical barriers to aphid feeding were evaluated by
14 nymph survival. Reduction in palatability of phloem sap was evaluated by nymph development
15 and mean relative growth rate. Electrical penetration graph recordings of feeding behaviour were
16 performed in order to localise aphid resistant factors. Nymph development was significantly longer
17 on AO-1012-29-3A compared to Kabulangeti despite the fact that there were significantly fewer
18 glandular trichomes on this line. The variety AO-1012-29-3A can be used in genetic improvement
19 of common bean for aphid resistance.

20 **Keywords**

21 Nymph development, mutagenesis, host plant resistance, cross resistance, plant breeding.

22

23 1 | INTRODUCTION

24 Common bean (*Phaseolus vulgaris* L.) is an important staple crop widely grown across
25 sub-Saharan Africa (De Jager, Borgonjen-Van Den Berg, Giller, & Brouwer, 2019; Ronner,
26 Descheemaeker, Almekinders, Ebanyat, & Giller, 2018). Beans are extremely nutritious as they
27 are rich in protein (Buruchara et al., 2011; Snapp, Rahmanian, & Batello, 2018), amino acids
28 (Mweetwa, Chilombo, & Gondwe, 2016; Ronner et al., 2018), vitamins, starch and fibre (Castro-
29 guerrero, Isidra-arellano, Mendoza-cozatl, González-guerrero, & Grusak, 2016). As well as being
30 nutritious, beans contain high quantities of polyphenolic compounds, such as flavonoids and
31 bioactive peptides, which offer health benefits by reducing risk to obesity and disease (Lin et al.,
32 2016; Ramírez-Jiménez, Reynoso-Camacho, Tejero, León-Galván, & Loarca-Piña, 2015).

1 Although common bean plays a vital role in improving food security, their economic importance
2 is also increasing as a method of generating income for smallholder farming communities
3 (Buruchara et al., 2011; Tembo, Namebo, Chanda, Kamfwa, & Munyinda, 2019). In Zambia,
4 common bean is the second most widely grown legume crop after groundnuts (*Arachis hypogea*
5 L.), with a total production area of 84,500 ha and average annual production of 52,300 t (Chapoto,
6 Chisanga, & Kabisa, 2019). However, common bean yields are low, varying from 300 to 500
7 kg/ha, compared to the yield potential of 2000 kg/ha (Kamfwa, Beaver, Cichy, & Kelly, 2018;
8 Tembo et al., 2019). Pests and diseases, particularly aphids and the viral pathogens they vector,
9 are among the major constraints to productivity (Worrall et al., 2015).

10 Black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), is one of the most
11 important common bean pests in sub-Saharan Africa (Abate & Ampofo, 1996; Esmaeili-
12 Vardanjani et al., 2013). While estimates in Zambia are not available, yield losses of up to 37 %
13 in Uganda have been attributed to black bean aphid (Mwangi, Deng, & Kamau, 2009). Crop
14 damage to susceptible cultivars by aphids occurs directly through feeding or indirectly through
15 virus transmission (Wainaina et al., 2019; Worrall et al., 2015). The ability of black bean aphids
16 to vector plant viruses, such as bean common mosaic virus (BCMV) has a greater impact on yield
17 than direct feeding damage (van Emden & Harrington 2017; Wainaina et al. 2019). Bean common
18 mosaic virus exists as a complex of strains, with seven pathotypes, belonging to the *Potyvirus*
19 genus in the *Potyviridae* family and is responsible for serious economic losses in common bean
20 (Feng, Poplawsky, Nikolaeva, Myers, & Karasev, 2014). This virus complex is "stylet borne" and
21 non-persistently transmitted between plants during feeding (Flores-Estévez, Acosta-Gallegos, &
22 Silva-Rosales, 2007; Wainaina et al., 2019; Worrall et al., 2015).

23 Several strategies are used in Zambia to control aphids on common bean crops, but each
24 has limited efficacy. Early planting is one cultural measure used to prevent susceptible seedlings
25 coinciding with high aphid populations soon after the cropping season begins (Musenga et al.,
26 2016). Another widely used measure is intercropping common bean with cereal crops like maize,
27 which has been found to reduce aphid colonisation of common bean plants (Ogenga-Latigo,
28 Baliddawa, & Ampofo, 1993). Foliar application of synthetic insecticides such as deltamethrin,
29 cypermethrin, lambda-cyhalothrin and thiamethoxam early in the season can also help to reduce
30 aphid colonisation of seedlings (Musenga et al., 2016). The use of synthetic insecticides,
31 however, may be detrimental to pollinators and natural enemies (Desneux, Decourtye, &
32 Delpuech, 2006) as well as human health (Carvalho, 2017; Kim, Kabir, & Jahan, 2017). Black
33 bean aphid is also known to have developed resistance to certain synthetic insecticides (e.g.,

carbamates and organophosphates), further reducing the reliability of chemical control (van Emden & Harrington, 2017). In addition to environmental and health concerns, high costs and limited availability further prevents widespread use of synthetic insecticides by resource poor farmers (Souleymane, Ova Aken, Fatokun, & Alabi, 2013). Therefore, there is an urgent need for cost-effective and sustainable alternatives to manage black bean aphid on common bean.

Developing common bean cultivars resistant to aphid pests could provide a sustainable, environmentally friendly and cost effective option for their management (Miklas, Kelly, Beebe, & Blair, 2006; Mwangi et al., 2009). 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 (Nalam, Louis, & Shah, 2019; Züst & Agrawal, 2016). 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 & Bonning, 2012). Protease inhibitors interfere with protease function in herbivorous insects and inhibit protein metabolism (Zhu-Salzman & Zeng, 2015). These anti-aphid plant traits may be expressed either constitutively or induced by feeding (Smith & Chuang, 2014; Westwood & Stevens, 2010).

To successfully breed resistant cultivars, sources of resistance are needed. Such resistance sources could include existing cultivars, wild relatives of crops, germplasm collections or induced mutations (Olasupo, Ilori, Forster, & Bado, 2018; Omoigui et al., 2017). Mutations can be induced by exposing plant propagules to physical or chemical mutagens that cause DNA changes, resulting in altered traits of treated plants (Mba, Afza, Bado, & Jain, 2010; Novak & Brunner, 1992). Such induced mutations also often produce genes or alleles not present in the natural population, increasing the chances of generating novel resistance traits (Novak & Brunner, 1992). Mutants 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). Selected examples of legume cultivars developed through induced mutations include high protein cowpea (*Vigna unguiculata* L.) (Adekola & Oluleye, 2007), drought tolerant cowpea (De Ronde & Spreeth, 2007), high yielding cowpea (Horn, Shimelis, & Laing, 2015) and early maturing common bean (Tulmann Neto et al., 2011).

Despite the economic importance of aphids in common bean, previous efforts to identify resistant genotypes have been limited. Identification of aphid resistant genotypes could support genetic enhancement of common bean for aphid resistance and mitigate the yield losses associated with aphids. Differential responses of aphids to plants based on life table parameters provide a reliable basis for identifying resistant genotypes including the mechanisms mediating resistance (Nalam et al., 2018; Obopile & Ositile, 2010). This study aimed to identify aphid resistant genotypes from selected varieties and mutants as well as any mechanisms mediating resistance. Specifically, deterrence to aphid was assessed by measuring settling preference. Physical barriers to aphid feeding were assessed by nymph survival while reduction in palatability of phloem sap was evaluated by nymph development and mean relative growth rate (MRGR). Electrical penetration graph (EPG) recordings of aphid feeding behaviour were performed to localise plant resistance factors.

2 | MATERIALS AND METHODS

2.1 | Common bean genotypes and experimental design

A total of eleven common bean genotypes were evaluated for aphid resistance (Table 1). Of these genotypes five were selected from the Andean Diversity Panel (ADP) (Cichy et al., 2015), four were mutant genotypes, one (Carioca) is a parent of the mutant lines, and one (AO-1012-29-3-3A) (AO) is a released variety. The genotypes selected from the ADP included Rozi Koko (ADP 1), Mwezi Moja (ADP 466), Majesty (ADP 684), and KK 25 (ADP 765). The ADP genotypes were selected based on their agronomic traits (ADP 1 and ADP 684), and anecdotal evidence on their resistance to pests such as weevils (ADP 765) and bean stem maggot (ADP 466). AO is a determinate dark red kidney variety that was developed, and released cooperatively by Sokoine University of Agriculture, Oregon State University, USDA-ARS and the University of Puerto Rico (Kusolwa et al., 2016). AO is resistant to common bean weevil (*Acanthoscelides obtectus*) (Kamfwa et al., 2018; Kusolwa et al., 2016). In addition, AO is resistant to Bean Common Mosaic Virus (Kusolwa et al., 2016) and some races of anthracnose (Mungalu et al., 2020). The commercial variety Kabulangeti (KAB), which is widely grown in Zambia, and Carioca (CA), a parent for the mutants were used as checks.

Two seeds of each genotype were sown in plastic pots (diameter and height: 9 cm) containing potting soil (John Innes No. 2, J. Arthur Bower's, Westland Horticulture Limited, Cheshire, UK) and placed in an insect proof mesh cage 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 ten days old (BBCH growth stage 11-12) (Lancashire et al., 1991), when plants were thinned to leave one seedling per pot. Irrigation was done by adding water to trays twice weekly throughout the study period. Twelve-day old common bean plants (BBCH growth stage 12) were used for the nymph development, settling preference, nymph survival, mean relative growth rate (MRGR) and feeding behaviour experiments. Twelve-day old plants were used in bioassays to match the aphid susceptible growth stage (Esmaeili-Vardanjani et al., 2013). Plants for the trichome density experiment were sown and thinned as described above but cultivated in an insect rearing tent within a glasshouse at 20 °C and 60 % relative humidity with an 18:6 photoperiod. Approximately 15 days after germination, a 1 m long stake was inserted into each pot (9 cm depth) to provide support to the growing bean plants. Plants were allowed to grow for approximately 28 days (BBCH growth stage 16+) to match the timing of the trichome study.

2.2 | Aphid culture and age-synchronised cohort production

A stock culture of black bean aphids (*Aphis fabae* Scopoli) was reared on field bean seedlings (*Vicia faba* cv. Tundra) in an insect proof mesh cage within a controlled environment room (Fitotron) maintained at 20 °C and 60 % relative humidity with a 16:8 photoperiod. The culture was maintained by transferring aphids onto new field bean seedlings weekly throughout the study period.

To produce a cohort of age-synchronised apterous adult aphids for nymph development, nymph survival, MRGR and feeding behaviour experiments, two to five apterous adult aphids were transferred onto individual broad bean seedlings within an insect proof cage. After 24 hours, adult aphids were removed from the plants using a 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, 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).

To produce winged adult aphids for the settling preference experiment, ten to fifteen apterous adult aphids were transferred onto individual 2-week-old field bean seedlings within an insect proof mesh cage. After 24 hours, adult aphids were removed from the plants using a paintbrush to leave only first instar nymphs. As described above, each plant was covered with a

fine light-transmitting mesh bag. Development of winged aphids was stimulated by the higher population of nymphs on seedlings and consequent rapid deterioration of plant nutrition quality (Blackman & Eastop, 2000). Plant nutritional quality was further reduced by irrigating bean plants only once per week. Field bean seedlings were maintained in a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod until the majority of nymphs moulted into winged adults (approximately seven to eight days).

2.3 | Assessment of nymph development

Development of black bean aphid nymphs was assessed using a procedure adapted from Soffan and Aldawood (2014). Using a paint brush, age-synchronised adult apterous aphids were individually placed onto common bean plant leaves in an insect proof mesh cage 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 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. Development through the immature nymph life cycle stages was monitored by the presence of exuviae, which were removed using a paintbrush and the date recorded. The total number of days between birth and adult emergence was also recorded simultaneously for each bean genotype within a replicate. Ten plants from each genotype were tested in this bioassay.

2.4 | Determination of trichome density

Glandular and hooked trichome density on common bean leaves was determined using a procedure adapted from Dahlin et al. (1992). A fully expanded middle leaflet was excised using a pair of scissors from the third trifoliate on each bean plant. Using a surgical blade, a 1 cm^2 section of leaf was removed from between the lateral veins at the widest region of the leaflet. Leaf sections were affixed onto scanning electron microscope (SEM) stubs using a double-sided adhesive tape, with the lower leaf surface facing up. Only trichomes on the lower leaf surface were considered in this study as it is the primary feeding site for black bean aphid (Prado & Tjallingii, 1997). Leaf samples were placed in a SEM specimen holder and dried in a desiccator for 48 hours. Dry leaf samples were then coated with a gold film using a sputter coater (Edwards S 150, Edwards High Vacuum, Crawley, Sussex, England). Hooked and glandular trichomes were counted and

photographed using the scanning electron microscope (Cambridge Stereoscan 200, Cambridge Instruments Ltd, Cambridge, UK). Four plants from each common bean genotype were evaluated.

2.5 | Settling preference of aphids on bean genotypes

Settling preference of black bean aphid was evaluated as described by Laamari et al. (2008) and Kamphuis et al. (2012). A single plant of each genotype was placed into a cage within a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod. Plants were placed in a circular pattern such that the pots were equidistant from each other and spaced as far apart as possible (approximately 15 cm) to prevent leaves of adjacent plants touching one another. A plastic 90 mm Petri dish containing a cohort of 120 age-synchronised alate aphids was placed at the centre of the cage, approximately 20 cm from each plant. Aphids were allowed to select plants on which to land and settle for a period of 72 hours. The number of aphids settling on each plant were recorded at 24, 48 and 72 hours after being released. Numbers of aphids settling on bean plants 24 hours after release indicated whether immediate deterrent factors (i.e. trichomes) were present or absent in bean genotypes while numbers of aphids recorded from 24 to 72 hours (through 48 hours) provided information whether the numbers of aphids settling on plants increased or decreased. The position of each genotype in the cage was randomly allocated in each replicate. Ten plants from each genotype were tested in this bioassay.

2.6 | Assessment of nymph survival

Survival of black bean aphid nymphs was as described by Obopile & Ositile (2010). Using a paintbrush, two age-synchronised apterous adult aphids were placed on each common bean plant in an insect proof mesh cage within a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod. After 24 hours, aphids were removed from plants using a paintbrush, leaving ten first instar nymphs per plant. Each plant was covered with a fine light-transmitting mesh bag and returned to the controlled environment room. Nymph survival was estimated as the number of aphids found on each plant after seven days, expressed as a proportion of the initial count on each bean plant. Ten plants from each genotype were tested in this bioassay.

2.7 | Mean relative growth rate of nymphs on bean genotypes

Mean relative growth rate of aphids was evaluated as described by van Emden & Bashford (1969). Using a paintbrush, two age-synchronised apterous adult aphids were placed on each common bean plant in an insect proof mesh cage within a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod. After 24 hours, all aphids were removed except ten first instar nymphs per plant which were weighed using a microbalance to record the initial mean weight. After weighing, nymphs were placed back onto respective plants and covered with a fine light-transmitting mesh bag. Plants were maintained in the controlled environment room for five days, when a single nymph from each plant was re-weighed to record the final weight. Mean relative growth rate was calculated using:

$$MRGR\ (\mu g/\mu g/day) = \frac{(\log(W_2) - \log(W_1))}{(t_2 - t_1)}$$

Where W_1 = initial mean weight of nymphs, W_2 = weight of a single nymph after five days and (t_2 – t_1) = period (days) between the first (t_1) and final weighing (t_2) (Castle & Berger, 1993). Ten plants from each genotype were tested in this bioassay.

2.8 | Monitoring of aphid feeding behaviour

Direct-current (DC) electrical penetration graph (EPG) recording was used to monitor probing and feeding behaviour of apterous adult aphids (Tjallingii, 1978). Since mutant common bean genotypes did not have a negative biological effect on aphids in preceding experiments (nymph development, settling preference, nymph survival and MRGR), only breeding lines were subjected to EPG recording. 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) 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. A total of fourteen successful recordings were carried out for individual aphids

feeding on each bean 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 (1988). Annotated waveforms were transformed into time-series data using the Excel macro software developed by Sarria et al. (2009).

2.9 | Experimental design and data analysis

Due to the homogeneity of environmental conditions in the controlled environment room and glasshouse as well as potting soil, 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. Normality of distribution was 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). Data that was neither normally distributed nor with homogenous variance, such as for settling preference and feeding behaviour, 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 were analysed with Kruskal-Wallis rank-sum tests. For each experiment the common bean varieties and corresponding mutation derived genotypes were analysed separately, except for the settling preference experiment where all varieties and mutant genotypes were analysed together.

3 | Results

Aphid performance bioassays were undertaken to assess the relative resistance of common bean genotypes in comparison with susceptible commercial cultivars. Among the parameters measured, significant differences in aphid performance were observed on nymph

1 development, trichome density and feeding behaviour (phloem ingestion). No significant
2 differences were detected for settling preference, nymph survival and MRGR.

3

4 **3.1 | Assessment of nymph development**

5 Nymph development on common bean varieties ranged from 9.4 (ADP 684) to 13.6 (AO)
6 days with a mean of 10.8 days. Significant differences in nymph development were detected
7 between common bean varieties (one-way ANOVA: $F = 12.58$, $df = 5$, $P = 0.001$) (Figure 1A).
8 Nymph development was significantly longer on AO-1012-29-3A compared to the commercial
9 variety (KAB) (Holm-Sidak test, $P = 0.006$). Nymph development on mutant lines ranged from
10 11.3 (CA 3) to 13.7 (CA 38) days with a mean of 12.4 days. Significant differences in nymph
11 development were observed between mutant lines (one-way ANOVA: $F = 4.26$, $df = 4$, $P = 0.005$).
12 However, none of the mutant lines differed significantly from the parent (Holm-Sidak test, $P >$
13 0.05) (Figure 1B).

14

15 **3.2 | Determination of trichome density**

16 Mean hooked trichome density on common bean varieties ranged from 9 (AO) to 39.7
17 (ADP 466) with a mean of 21.8. Significant differences were detected between common bean
18 varieties (one-way ANOVA: $F = 25.59$, $df = 5$, $P = 0.001$). Genotypes ADP 466 (Holm-Sidak test,
19 $P < 0.0001$) and ADP 765 (Holm-Sidak test, $P = 0.002$) had 26.3 and 17.0 more hooked trichomes
20 respectively compared to the commercial variety (KAB) (Figure 2A). Densities of hooked
21 trichomes in mutant derived genotypes ranged from 12.5 (CA 24) to 21.5 (CA) with a mean of
22 15.8. Significant differences were detected between mutation derived genotypes (one-way
23 ANOVA: $F = 3.69$, $df = 4$, $P = 0.03$) (Figure 2B). Genotype CA 24 had lower numbers of hooked
24 trichomes compared to the parent (Holm-Sidak test, $P = 0.03$) (Figure 2B).

25 In the case of glandular trichomes, densities on common bean varieties ranged from 0.25
26 (AO) to 3.75 (ADP 684 and KAB) with a mean of 2.4. Significant differences were observed
27 between bean varieties (one-way ANOVA: $F = 7.92$, $df = 5$, $P < 0.001$). Genotypes ADP 1 (Holm-
28 Sidak test, $P = 0.049$) and AO (Holm-Sidak test, $P = 0.001$) had 2.3 and 3.5 less glandular
29 trichomes respectively compared to the commercial variety (KAB) (Figure 2C). No significant
30 differences were observed between mutation derived genotypes and the parent (one-way
31 ANOVA: $F = 2.84$, $df = 4$, $P > 0.05$) (Figure 2D).

3.3 | Settling preference of aphids on bean genotypes

The number of alate aphids settling on plants did not differ significantly between the common bean genotypes (one-way ANOVA: F = 0.82, df = 10, P = 0.59) 24 hours after being released (Table 2). Alate aphid numbers did not change significantly either 48 hours (one-way ANOVA: F = 1.03, df = 10, P = 0.38) or 72 hours (one-way ANOVA: F = 1.03, df = 10, P = 0.40) after release (Table 2).

3.4 | Assessment of nymph survival

Nymph survival on mutation derived genotypes ranged from 33 (CA) to 70 % (CA 38) with a mean of 49.4 %. Significant differences were observed between mutation derived genotypes and the parent (one-way ANOVA: F = 7.09, df = 4, P = 0.0002). On genotype CA 38, 37 % more aphids survived compared to the parent (CA) (Holm-Sidak test, P = 0.0001) (Figure 3B). In the case of common bean varieties, there were no significant differences in nymph survival between common bean varieties and the commercial variety (KAB) (one-way ANOVA: F = 1.42, df = 5, P = 0.23) (Figure 3A).

3.5 | Mean relative growth rate of nymphs on bean genotypes

When MRGR was assessed, significant differences were detected between common bean varieties (one-way ANOVA: F = 2.52, df = 5, P = 0.04). However, none of the bean varieties were differed significantly from the commercial variety. In the case of mutation derived genotypes, no significant differences were detected between mutant genotypes (one-way ANOVA: F = 1.55, df = 4, P = 0.20).

3.6 | Monitoring of aphid feeding behaviour

Total duration of phloem ingestion ranged from 2.5 (ADP 1) to 22.4 minutes (ADP 684) with a mean of 6.9 minutes. Significant differences in the phloem ingestion were observed between the common bean varieties and the commercial variety (KAB) (one-way ANOVA: F = 4.39, df = 5, P = 0.002) (Figure 4F). Aphids fed on ADP 684 for 18.2 more minutes compared to

the commercial variety (Holm-Sidak test, P = 0.01) (Figure 4F). There were no significant differences between bean varieties in the duration of the non-probing phase (Kruskal-Wallis: X^2 = 7.96, df = 5, P = 0.15) (Figure 4A), period to first probe from beginning of EPG recording (one-way ANOVA: F = 1.07, df = 5, P = 0.28) (Figure 4B), pathway phase (one-way ANOVA: F = 1.06, df = 5, P = 0.39) (Figure 4C), period from first probe to phloem ingestion (one-way ANOVA: F = 0.79, df = 5, P = 0.55) (Figure 4D), and phloem salivation (one-way ANOVA: F = 0.72, df = 5, P = 0.51) (Figure 4E).

4 | DISCUSSION

A greater understanding of the mechanisms mediating plant-aphid interactions is an important preliminary step in breeding aphid resistant crop varieties. This study demonstrated that some biological parameters of black bean aphid were significantly influenced by common bean genotypes. Nymph development was significantly longer on AO compared to the commercial cultivar KAB, indicating a level of host plant resistance (Obopile & Ositile, 2010). Longer nymph development may suggest reduced nutritional quality of phloem sap, which could have resulted in poor nourishment and therefore slower development (Leybourne et al., 2019). Indeed, although statistically insignificant, EPG analysis indicated reduced phloem feeding by aphids on AO compared to aphids on KAB. This adverse effect of AO on aphid nymph development may suggest that reduced palatability is the major resistant trait against the cowpea aphid. Characterisation of amino acids and defense compounds in AO should be considered in future studies in order to establish the modality of resistance.

Studies in other legume-aphid systems have also pointed to the importance of mesophyll- and phloem-based resistance traits (Kamphuis et al., 2012; Leybourne et al., 2019). Resistance to pea aphid (*Acyrthosiphon pisum* Harris) in pea cultivars (*Pisum sativum* L.), for example, has been partly attributed to imbalances in essential amino acid composition in the phloem sap (Sandström & Pettersson, 1994). The variety AO is resistant to common bean weevil (*A. obtectus*) (Kusolwa et al., 2016) and this resistance has been attributed to insecticidal activity of three proteins including arcelins, phytohemagglutinin and alpha-amylase (Kusolwa & Myers, 2011). There is a possibility of cross-resistance to black bean aphid since the aphicidal effects of lectins and protease inhibitors are well known (Nalam et al., 2019). However, biochemical characterisation of essential amino acids and their role in cross-resistance to common bean weevil and black bean aphid, should be considered in future studies. Results presented here

reflect the interaction between a range of plant lines but only a single aphid clone. As such it would be useful to repeat this work using other aphid clones in order to establish if the results reported here are consistent for a wider range of aphid genotypes.

Common bean plants are known to possess trichomes that serve as physical defences against aphid attack (Xing et al., 2017). Hooked trichomes, for example, may trap or impale aphids while glandular trichomes may exude toxic compounds or adhesive fluids that trap insects (Saska et al., 2020). In this study, significantly fewer glandular trichomes were detected on lines ADP 1 and AO compared to the commercial cultivar. Since low glandular trichome densities did not reflect an increased survival of aphids on ADP 1 and AO, it is likely that glandular trichomes were not a primary modality of resistance to black bean aphid in these genotypes. Nymph survival is among other factors often associated with high trichome density (Saska et al., 2020). This study showed that nymph survival did not vary significantly between the bean genotypes. Indeed, no significant differences were detected in the number of hooked trichomes between the bean genotypes that usually affect nymph survival on common bean (Xing et al., 2017). Given the presence of other resistance mechanisms, low trichome density on bean genotypes would benefit the performance of natural enemies which could complement aphid control (Riddick & Simmons, 2014). Although the adverse impacts of hooked trichomes to black bean aphid are well studied for common bean (Xing et al., 2017), the impact of glandular trichomes is poorly understood and would warrant further study.

Alate aphids often use visual and volatile cues to locate their host plants (Döring, 2014; Powell, Tosh, & Hardie, 2006; Webster et al., 2008). Observations of black bean aphid settling behaviour showed that their preference for the tested common bean genotypes did not vary significantly between the genotypes 24 hours after release, suggesting similarities in aphid host location cues. Between 24 and 72 hours, there was little movement of aphids between plants, indicating lack of host preference. Black bean aphid is known to discriminate host plants based on colour and semiochemicals. For example, using a wind tunnel and monochromatic light, Hardie (1989) showed that black bean aphids were preferentially attracted to the green region of the spectrum, indicating a preference for green coloured plants. In olfactometer experiments, Nottingham et al. (1991) showed that black bean aphids were able to discriminate between cultivars ("Sutton Dwarf" and "Tick Bean") of field bean, suggesting the role of semiochemicals in host location.

Nymph MRGR on common bean breeding lines did not vary significantly when compared to the commercial cultivar. However, the general trend showed lowest and highest weight gain of

nymphs on AO and ADP 684, respectively, which may be a consequence of phloem nutrition quality of these genotypes. Feeding analysis by EPG indicated significantly prolonged phloem feeding on ADP 684 compared to the commercial cultivar, suggesting higher susceptibility through reduced resistance in the phloem. Mean relative growth rate is often a good predictor of aphid performance since lower weight gains are associated with reduced fecundity and population growth (Dixon & Wratten, 1971). Based on the trend, lower MRGR of black bean aphid on AO may be attributed to reduced food quality (Obopile & Ositile, 2010).

This study showed that black bean aphid nymphs feeding on AO developed more slowly than other lines tested or KAB. Although not significant, AO further reflected reduced aphid weight gain and phloem feeding. Lower glandular trichome density was also recorded on AO, which could benefit natural enemy performance and consequently enhance biological control of aphids (Riddick & Simmons, 2014). AO is therefore a promising variety that should be further evaluated for useful genetic attributes that may be used to develop aphid resistant common bean varieties. On the other hand, mutagenesis did not generate resistance to black bean aphid in the tested mutant lines. Future studies should consider screening a wider range of mutants in order to increase chances of finding aphid resistant genotypes. However, based on other grower preferred attributes (i.e., seed size and colour) associated with the mutants tested, further studies are needed to establish if mutagenesis generated other useful traits that may be beneficial to aphid natural enemies.

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 common bean seed used in this study from the Department of Plant Science at the University of Zambia is greatly acknowledged.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

REFERENCES

1 Abate, T., & Ampofo, J. K. O. (1996). Insect Pests of Common Bean in Africa: Their Ecology
2 and Management. *Annual Review of Entomology*, *41*(1), 45–73.
3 https://doi.org/10.1146/annurev.ento.41.1.45

4 Adekola, O. F., & Oluleye, F. (2007). Influence of mutation induction on the chemical
5 composition of cowpea Vigna unguiculata (L.) Walp. *African Journal of Biotechnology*,
6 *6*(18), 2143–2146.

7 Blackman, R. L., & Eastop, V. F. (2000). *Aphids on the World's Crops: An Identification and*
8 *Information Guide* (Second Edi). Chichester: John Wiley & Sons.

9 Boness, M., & Unterstenhöfer, G. (1974). Insecticide resistance in aphids. *Zeitschrift Für*
10 *Angewandte Entomologie*, *77*, 1–19.

11 Buruchara, R., Chirwa, R., Sperling, L., Mukankusi, C., Rubyogo, J. C., & Muthoni, R. (2011).
12 Development and delivery of bean varieties in Africa: The Pan-Africa bean research
13 alliance (PABRA) model. *African Crop Science Journal - A Journal of Tropical Crop*
14 *Science and Production*, *19*(4), 227–245.

15 Carvalho, F. P. (2017). Pesticides, environment, and food safety. *Food and Energy Security*,
16 *6*(2), 48–60. https://doi.org/10.1002/fes3.108

17 Castle, S. J., & Berger, P. H. (1993). Rates of growth and increase of Myzus persicae on virus-
18 infected potatoes according to type of virus-vector relationship. *Experimentalis et Applicata*,
19 *69*(1), 51–60.

20 Castro-guerrero, N. A., Isidra-arellano, M. C., Mendoza-cozatl, D. G., González-guerrero, M., &
21 Grusak, M. A. (2016). *Common Bean : A Legume Model on the Rise for Unraveling*
22 *Responses and Adaptations to Iron , Zinc , and Phosphate Deficiencies*. *7*(May), 1–7.
23 https://doi.org/10.3389/fpls.2016.00600

24 Chapoto, A., Chisanga, B., & Kabisa, M. (2019). *Zambia Agriculture Status Report 2018*.
25 Lusaka.

26 Chougule, N. P., & Bonning, B. C. (2012). Toxins for transgenic resistance to hemipteran pests.
27 *Toxins*, *4*(6), 405–429. https://doi.org/10.3390/toxins4060405

28 Dahlin, R. M., Brick, M. A., & Ogg, J. B. (1992). Characterization and density of trichomes on
29 three common bean cultivars. *Economic Botany*, *46*(3), 299–304.
30 https://doi.org/10.1007/BF02866628

1 De Jager, I., Borgonjen-Van Den Berg, K. J., Giller, K. E., & Brouwer, I. D. (2019). Current and
2 potential role of grain legumes on protein and micronutrient adequacy of the diet of rural
3 Ghanaian infants and young children: Using linear programming. *Nutrition Journal*, *18*(1),
4 1–16. https://doi.org/10.1186/s12937-019-0435-5

5 De Ronde, J. A., & Spreeth, M. H. (2007). Development and evaluation of drought resistant
6 mutant germ-plasm of Vigna unguiculata. *Water SA*, *33*(3 SPECIAL EDICTION), 381–386.
7 https://doi.org/10.4314/wsa.v33i3.180600

8 Desneux, N., Decourtye, A., & Delpuech, J.-M. (2006). The Sublethal Effects of Pesticides on
9 Beneficial Arthropods. *Annual Review of Entomology*, *52*(1), 81–106.
10 https://doi.org/10.1146/annurev.ento.52.110405.091440

11 Dixon, A. F. G., & Wratten, S. D. (1971). Laboratory studies on aggregation, size and fecundity
12 in the black bean aphid, Aphis fabae Scop. *Bulletin of Entomological Research*, *61*(01), 97.
13 https://doi.org/10.1017/s0007485300057485

14 Döring, T. F. (2014). How aphids find their host plants, and how they don't. *Annals of Applied
15 Biology*, *165*(1), 3–26. https://doi.org/10.1111/aab.12142

16 Esmaeili-Vardanjani, M., Askarianzadeh, A., Saeidi, Z., Hasanshahi, G. H., Karimi, J., &
17 Nourbakhsh, S. H. (2013). A study on common bean cultivars to identify sources of
18 resistance against the black bean aphid, Aphis fabae Scopoli (Hemiptera: Aphididae).
19 *Archives of Phytopathology and Plant Protection*, *46*(13), 1598–1608.
20 https://doi.org/10.1080/03235408.2013.772351

21 Feng, X., Poplawsky, A. R., Nikolaeva, O. V., Myers, J. R., & Karasev, A. V. (2014).
22 Recombinants of bean common mosaic virus (bcmv) and genetic determinants of bcmv
23 involved in overcoming resistance in common bean. *Phytopathology*, *104*(7), 786–793.
24 https://doi.org/10.1094/PHYTO-08-13-0243-R

25 Flores-Estévez, N., Acosta-Gallegos, J. A., & Silva-Rosales, L. (2007). Bean common mosaic
26 virus and Bean common mosaic necrosis virus in Mexico . *Plant Disease*, *87*(1), 21–25.
27 https://doi.org/10.1094/pdis.2003.87.1.21

28 Hardie, J. (1989). Spectral specificity for targeted flight in the black bean aphid, Aphis fabae.
29 *Journal of Insect Physiology*, *35*(8), 619–626.

30 Horn, L., Shimelis, H., & Laing, M. (2015). *Participatory appraisal of production constraints ,*

1 *preferred traits and farming system of cowpea in the northern Namibia : implications for*
2 *breeding*. *38*(5), 691–700. https://doi.org/10.18805/lr.v38i5.5952

3 Jaouannet, M., Rodriguez, P. A., Thorpe, P., Lenoir, C. J. G., Macleod, R., Escudero-Martinez,
4 C., & Bos, J. I. B. (2014). Plant immunity in plant-aphid interactions. *Frontiers in Plant*
5 *Science*, *5*(DEC), 1–10. https://doi.org/10.3389/fpls.2014.00663

6 Kamfwa, K., Beaver, J. S., Cichy, K. A., & Kelly, J. D. (2018). QTL Mapping of Resistance to
7 Bean Weevil in Common Bean. *Crop Science*, *58*(6), 2370–2378.
8 https://doi.org/10.2135/cropsci2018.02.0106

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

11 Kim, K. H., Kabir, E., & Jahan, S. A. (2017). Exposure to pesticides and the associated human
12 health effects. *Science of the Total Environment*, *575*, 525–535.
13 https://doi.org/10.1016/j.scitotenv.2016.09.009

14 Kusolwa, P. M., & Myers, J. R. (2011). Seed storage proteins ARL2 and its variants from the
15 apalocus of wild bean G40199 confers resistance to Acanthocellides obtectus when
16 expressed in common beans. *African Crop Science Journal*, *19*(4), 255–265.

17 Laamari, M., Khelfa, L., & Cœur d'Acier, A. (2008). Resistance source to cowpea aphid (Aphis
18 craccivora Koch) in broad bean (Vicia faba L .) Algerian landrace collection. *African*
19 *Journal of Biotechnology*, *7*(14), 2486–2490.

20 Lancashire, P. D., Bleiholder, H., Boom, T. V. D., Langelüddeke, P., Stauss, R., Weber, E., &
21 Witzenberger, A. (1991). A uniform decimal code for growth stages of crops and weeds.
22 *Annals of Applied Biology*, *119*(3), 561–601. https://doi.org/10.1111/j.1744-
23 7348.1991.tb04895.x

24 Leybourne, D. J., Valentine, T. A., Robertson, J. A. H., Pérez-Fernández, E., Main, A. M.,
25 Karley, A. J., & Bos, J. I. B. (2019). Defence gene expression and phloem quality
26 contribute to mesophyll and phloem resistance to aphids in wild barley. *Journal of*
27 *Experimental Botany*, *70*(15), 4011–4026. https://doi.org/10.1093/jxb/erz163

28 Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., … Chen, S. (2016). An overview of plant
29 phenolic compounds and their importance in human nutrition and management of type 2
30 diabetes. *Molecules*, *21*(10), 1–19. https://doi.org/10.3390/molecules21101374

Mba, C., Afza, R., Bado, S., & Jain, S. M. (2010). Induced mutagenesis in plants using physical and chemical agents. In *Plant cell culture: essential methods* (pp. 111–130).

Miklas, P. N., Kelly, J. D., Beebe, S. E., & Blair, M. W. (2006). Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. *Euphytica*, *147*(1–2), 105–131. https://doi.org/10.1007/s10681-006-4600-5

Musenga, C., Mwaba, D., Kilubi, V., Bwalya, K., Mweembe, J., Siame, B., … Ntenga, I. (2016). Pest Management Decision Guide: Green and Yellow List, Cowpea aphids on cowpeas. Zambia Agricultural Research Institute (ZARI)-Plantwise. Retrieved from CABI website: https://www.plantwise.org/KnowledgeBank/pmdg/20177800703

Mwangi, S. N., Deng, A. L., & Kamau, A. W. (2009). Response of Kenyan varieties of common bean, Phaseolus vulgaris L., to infestation by Aphis fabae Scopoli. *African Entomology*, *16*(2), 196–202. https://doi.org/10.4001/1021-3589-16.2.196

Mweetwa, A. M., Chilombo, G., & Gondwe, B. M. (2016). *Nodulation , Nutrient Uptake and Yield of Common Bean Inoculated with Rhizobia and Trichoderma in an Acid Soil*. *8*(12), 61–71. https://doi.org/10.5539/jas.v8n12p61

Nalam, V. J., Louis, J., Patel, M., & Shah, J. (2018). Arabidopsis-green peach aphid interaction: Rearing the insect, no-choice and fecundity assays, and electrical penetration graph technique to study insect feeding behavior. *Bio-Protocol*, *8*(15), 1–24. https://doi.org/10.21769/BioProtoc.2950

Nalam, V. J., Louis, J., & Shah, J. (2019). Plant defense against aphids, the pest extraordinaire. *Plant Science*, *279*(August), 96–107. https://doi.org/10.1016/j.plantsci.2018.04.027

Nottingham, S. F., Hardie, J., Dawson, G. W., Hick, A. J., Pickett, J. A., Wadhams, L. J., & Woodcock, C. M. (1991). Behavioral and electrophysiological responses of Aphids to host and nonhost plant volatiles. *Journal of Chemical Ecology*. https://doi.org/10.1007/BF01402946

Novak, F. J., & Brunner, H. (1992). Plant breeding: Induced mutation technology for crop improvement. *IAEA Bulletin*, *4*, 24–33.

Obopile, M., & Ositile, B. (2010). *Life table and population parameters of cowpea aphid , Aphis craccivora Koch (Homoptera : Aphididae) on five cowpea Vigna unguiculata (L . Walp .) varieties*. 9–14. https://doi.org/10.1007/s10340-009-0262-0

1 Ogenga-Latigo, M. W., Baliddawa, C. W., & Ampofo, J. K. O. (1993). Factors influencing the
2 incidence of the black bean aphid, Aphis fabae Scop., on common beans intercropped with
3 maize. *African Crop Science Journal*, *1*(1), 49–58.

4 Olasupo, F. O., Ilori, C. O., Forster, B. P., & Bado, S. (2018). Selection for Novel Mutations
5 Induced by Gamma Irradiation in Cowpea [Vigna unguiculata (L.) Walp.]. *International*
6 *Journal of Plant Breeding and Genetics*, *12*(1), 1–12.
7 https://doi.org/10.3923/ijpbg.2018.1.12

8 Omoigui, L. O., Ekeuro, G. C., Kamara, A. Y., Bello, L. L., Timko, M. P., & Ogunwolu, G. O.
9 (2017). New sources of aphids [Aphis craccivora (Koch)] resistance in cowpea germplasm
10 using phenotypic and molecular marker approaches. *Euphytica*, *213*(8), 178.
11 https://doi.org/10.1007/s10681-017-1962-9

12 Powell, G., Tosh, C. R., & Hardie, J. (2006). Host plant selection by aphids: Behavioral,
13 evolutionary, and applied perspectives. *Annual Review of Entomology*, *51*(22), 309–330.
14 https://doi.org/10.1146/annurev.ento.51.110104.151107

15 Prado, E., & Tjallingii, W. F. (1997). Effects of previous plant infestation on sieve element
16 acceptance by two aphids. *Entomologia Experimentalis et Applicata*, *82*(2), 189–200.
17 https://doi.org/10.1046/j.1570-7458.1997.00130.x

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

20 Ramírez-Jiménez, A. K., Reynoso-Camacho, R., Tejero, M. E., León-Galván, F., & Loarca-Piña,
21 G. (2015). Potential role of bioactive compounds of Phaseolus vulgaris L. on lipid-lowering
22 mechanisms. *Food Research International*, *76*(P1), 92–104.
23 https://doi.org/10.1016/j.foodres.2015.01.002

24 Riddick, E. W., & Simmons, A. M. (2014). Do plant trichomes cause more harm than good to
25 predatory insects? *Pest Management Science*, *70*(11), 1655–1665.
26 https://doi.org/10.1002/ps.3772

27 Ronner, E., Descheemaeker, K., Almekinders, C. J. M., Ebanyat, P., & Giller, K. E. (2018).
28 Farmers' use and adaptation of improved climbing bean production practices in the
29 highlands of Uganda. *Agriculture, Ecosystems and Environment*, *261*(January 2017), 186–
30 200. https://doi.org/10.1016/j.agee.2017.09.004

1 Sandström, J., & Pettersson, J. (1994). Amino acid composition of phloem sap and the relation
2 to intraspecific variation in pea aphid (Acyrthosiphon pisum) performance. *Journal of Insect*
3 *Physiology*, *40*(11), 947–955.

4 Saska, P., Skuhrovec, J., Tylová, E., Platková, H., Tuan, S. J., Hsu, Y. T., & Vítámvás, P.
5 (2020). Leaf structural traits rather than drought resistance determine aphid performance
6 on spring wheat. *Journal of Pest Science*, (0123456789). https://doi.org/10.1007/s10340-
7 020-01253-3

8 Smith, C. M., & Chuang, W. (2014). Plant resistance to aphid feeding : behavioral , physiological
9 , genetic and molecular cues regulate aphid host selection and feeding. *Pest Management*
10 *Science*, *70*(4), 528–540. https://doi.org/10.1002/ps.3689

11 Snapp, S., Rahmanian, M., & Batello, C. (2018). Pulse Crops for Sustainable Farms in Sub-
12 Saharan Africa. In *Pulse Crops for Sustainable Farms in Sub-Saharan Africa*. FAO.
13 https://doi.org/10.18356/6795bfaf-en

14 Soffan, A., & Aldawood, A. S. (2014). Biology and Demographic Growth Parameters of Cowpea
15 Aphid (Aphis craccivora) on Faba Bean (Vicia faba) Cultivars. *Journal of Insect Science*,
16 *14*(120), 1–10. https://doi.org/10.1673/031.014.120

17 Souleymane, A., Ova Aken, M. E., Fatokun, C. A., & Alabi, O. Y. (2013). Screening for
18 resistance to cowpea aphid (Aphis craccivora Koch) in wild and cultivated cowpea (Vigna
19 unguiculata L. Walp.) accessions. *International Journal of Science, Environment and*
20 *Technology*, *2*(4), 611–621.

21 Tembo, L., Namebo, M., Chanda, R., Kafwa, K., & Munyindaq, K. (2019). Genotypic Variation
22 for Response to Phosphorus Fertilization in Common Bean Mutants. *Canadian Journal of*
23 *Agriculture and Crops*, *4*(1), 11–16. https://doi.org/10.20448/803.4.1.11.16

24 Tulmann Neto, A., Ando, A., Figueira, A., Latado, R. R., Santos, P. C., Correa, L. S., … Ferreira
25 Filho, A. W. P. (2011). Genetic improvement of crops by mutation techniques in Brazil.
26 *Plant Mutation Reports*, *2*(3), 24–37.

27 van Emden, H. F., & Bashford, M. A. (1969). A comparison of the reproduction of Brevicoryne
28 brassicae and Myzus persicae in relation to soluble nitrogen concentration and leaf age
29 (leaf position) in the Brussels sprout plant. *Entomologia Experimentalis et Applicata*, *12*(3),
30 351–364.

van Emden, H. F., & Harrington, R. (2017). *Aphids as Crop Pests* (Second Edi). Oxfordshire: CABI.

Van Lenteren, J. C., & De Ponti, O. M. B. (1991). Plant-leaf morphology, host-plant resistance and biological control. *Insects-Plants '89. Proc. 7th Symposium on Insect-Plant Relationships, Budapest, 1989*, (August), 365–386.

Wainaina, J. M., Kubatko, L., Harvey, J., Ateka, E., Makori, T., Karanja, D., … Kehoe, M. A. (2019). Evolutionary insights of Bean common mosaic necrosis virus and Cowpea aphid-borne mosaic virus. *PeerJ*, *7*, e6297. https://doi.org/10.7717/peerj.6297

Webster, B., Bruce, T., Dufour, S., Birkemeyer, C., Birkett, M., Hardie, J., & Pickett, J. (2008). Identification of volatile compounds used in host location by the black bean aphid, Aphis fabae. *Journal of Chemical Ecology*, *34*(9), 1153–1161.

Westwood, J. H., & Stevens, M. (2010). *Resistance to Aphid Vectors of Virus Disease*. Cambridge: Elsevier Inc.

Worrall, E. A., Wamonje, F. O., Mukeshimana, G., Harvey, J. J. W., Carr, J. P., & Mitter, N. (2015). Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus: Relationships, Biology, and Prospects for Control. In *Advances in Virus Research* (Vol. 93, pp. 1–46). https://doi.org/10.1016/bs.aivir.2015.04.002

Xing, Z., Liu, Y., Cai, W., Huang, X., Wu, S., & Lei, Z. (2017). Efficiency of trichome-based plant defense in phaseolus vulgaris depends on insect behavior, plant ontogeny, and structure. *Frontiers in Plant Science*, *8*(November), 1–8. https://doi.org/10.3389/fpls.2017.02006

Zhu-Salzman, K., & Zeng, R. (2015). Insect Response to Plant Defensive Protease Inhibitors. *Annual Review of Entomology*, *60*(1), 233–252. https://doi.org/10.1146/annurev-ento-010814-020816

Züst, T., & Agrawal, A. A. (2016). Mechanisms and evolution of plant resistance to aphids. *Nature Plants*, *2*(January), 1–9. https://doi.org/10.1038/nplants.2015.206

TABLES

TABLE 1 List and category of common bean (*Phaseolus vulgaris* L.) genotypes used in the study.

Common bean line	Seed colour	Country of origin	Category
Rozi Koko (ADP 1)	Red-Mottled	Kenya	Variety
Mwezi Moja (ADP 466)	Purple	Kenya	Variety
Majesty (ADP 684)	Red Kidney	Canada	Variety
KK25 (ADP 765)	Red	Malawi	Landrace
AO 1012-29-3A	Red	-	Variety
Kabulangeti (KAB)	Purple	Zambia	Variety
CA 3	Brown-Mottled	Zambia	Mutant
CA 15	Brown-Mottled	Zambia	Mutant
CA 24	Brown-Mottled	Zambia	Mutant
CA 38	White	Zambia	Mutant
CA	Brown	Brazil	Parent line (for mutants)

1

2

3

4

5

6

7

8

9

10

11

12 **TABLE 2** Settling preference of adult winged aphid black bean aphid (*Aphis fabae* Scopoli) on

13 common bean genotypes 24, 48 and 72 hours after being released in the cage (SE = standard

error; n = 10). Common bean breeding lines and mutation derived genotypes were analysed together.

		No. aphids per plant (mean ± SE)		
Genotype	**Category**	**24 hrs**	**48 hrs**	**72 hrs**
Rozi Koko (ADP 1)	Variety	2.2 ± 0.4	2.4 ± 0.5	2.6 ± 0.5
Mwezi Moja (ADP 466)	Variety	6.9 ± 1.4	7.7 ± 1.5	7.3 ± 1.5
Majesty (ADP 684)	Variety	5.4 ± 1.7	5.2 ± 1.6	5.1 ± 1.4
KK25 (ADP 765)	Landrace	4.9 ± 1.4	4.9 ± 1.4	4.5 ± 1.3
AO 1012-29-3A	Variety	5.5 ± 1.6	6.1 ± 1.9	5.7 ± 1.9
Kabulangeti (KAB)	Variety	5.2 ± 1.6	5.7 ± 1.0	5.6 ± 0.9
CA 15	Mutant	6.3 ± 1.4	6.7 ± 1.9	5.5 ± 1.3
CA 24	Mutant	4.6 ± 1.1	5.2 ± 1.1	4.9 ± 1.1
CA 3	Mutant	8.0 ± 2.3	7.5 ± 2.0	6.7 ± 1.8
CA 38	Mutant	3.6 ± 0.8	3.4 ± 0.7	2.9 ± 0.5
CA	Parent line (for mutants)	3.8 ± 0.9	3.4 ± 0.9	3.0 ± 0.7
P-value		0.596	0.383	0.402

FIGURE LEGENDS

1 **FIGURE 1** Black bean aphid nymph development on (A) common bean breeding lines and (B)
2 mutation derived genotypes (*n* =10). Bars followed by different letters are significantly different
3 (Holm-Sidak post-hoc test). Among the common bean breeding lines, nymph development was
4 significantly longer on AO-1012-29-3A compared to the commercial variety Kabulangeti (Holm-
5 Sidak test, $P = 0.006$). Among mutation derived genotypes, none of the mutants differed
6 significantly from the parent (CA) (Holm-Sidak test, $P > 0.05$). Error bars represent standard error
7 of the mean (SE). Common bean breeding lines and corresponding mutation derived genotypes
8 were analysed separately. Genotype name; ADP 1 = Rozi Koko, ADP 466 = Mwezi Moja, ADP
9 684 = Majesty, ADP 765 = KK25

10

11 **FIGURE 2** Median densities and confidence intervals of (A) hooked trichomes on common bean
12 breeding lines (B) hooked trichomes on mutation derived genotypes (C) glandular trichomes on
13 common bean breeding lines and (D) glandular trichomes on mutation derived genotypes, on
14 lower leaf surfaces (*n* = 4). Among common bean breeding lines, ADP 765 (Holm-Sidak test, $P =$
15 0.002) as well as ADP 466 (Holm-Sidak test, $P < 0.0001$) had significantly higher numbers of
16 hooked trichomes compared to the commercial variety. For mutation derived genotypes, CA 24
17 had smaller numbers of hooked trichomes compared to the parent (Holm-Sidak test, $P = 0.03$).
18 For glandular trichomes on common bean breeding lines, AO-1012-29-3A (Holm-Sidak test, $P =$
19 0.001) and ADP 1 (Holm-Sidak test, $P = 0.049$) had fewer trichomes compared to the commercial
20 variety Kabulangeti. Common bean breeding lines and corresponding mutation derived
21 genotypes were analysed separately. Groups followed by different letters are significantly different
22 (Holm-Sidak post-hoc test), ns = non-significant differences between groups. Genotype name;
23 ADP 1 = Rozi Koko, ADP 466 = Mwezi Moja, ADP 684 = Majesty, ADP 765 = KK25

24

25 **FIGURE 3** Black bean aphid nymph survival on (A) common bean breeding lines and (B) mutation
26 derived genotypes (*n* = 10). Among the mutation derived genotypes, more aphids survived on CA
27 38 compared to the parent (CA) (Holm-Sidak test, $P = 0.0001$). Bars followed by different letters
28 are significantly different (Holm-Sidak post-hoc test). ns = non-significant differences among bars.
29 Error bars represent standard error of the mean (SE). Common bean varieties and corresponding
30 mutation derived genotypes were analysed separately. Genotype name; ADP 1 = Rozi Koko, ADP
31 466 = Mwezi Moja, ADP 684 = Majesty, ADP 765 = KK25

32

1 **FIGURE 4** Aphid probing and feeding behaviour on common bean breeding lines. (A) total time
2 of non-probing phase, (B) time to first probe from beginning of EPG recording, (C) total time of
3 pathway phase, (D) time from first probe to sustained phloem ingestion, (E) total time of phloem
4 salivation and, (F) total time of phloem ingestion (n = 14). In the case of phloem ingestion (4F),
5 aphids fed longer on ADP 684 compared to the commercial variety Kabulangeti (Holm-Sidak test,
6 P = 0.01), Common bean genotypes in figures A-F were analysed separately. Error bars represent
7 standard error of the mean (SE), Bars followed by different letters are significantly different (Holm-
8 Sidak post-hoc test), ns = non-significant differences among bars. Genotype name; ADP 1 = Rozi
9 Koko, ADP 466 = Mwezi Moja, ADP 684 = Majesty, ADP 765 = KK25

10

FIGURES

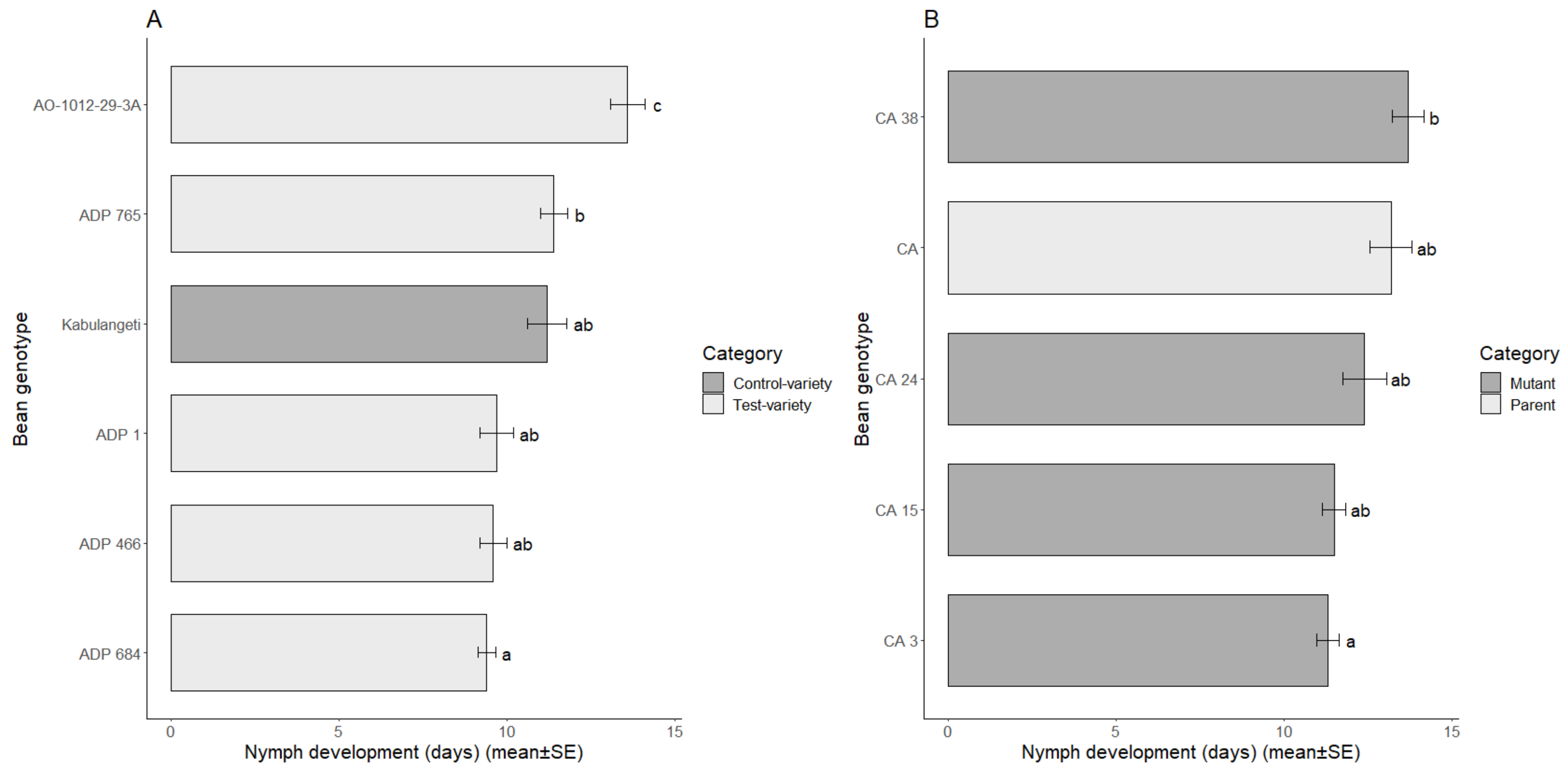

FIGURE 1 Black bean aphid nymph development on (A) common bean breeding lines and (B) mutation derived genotypes (*n* =10). Bars followed by different letters are significantly different (Holm-Sidak post-hoc test). Among the common bean breeding lines, nymph development was significantly longer on AO-1012-29-3A compared to the commercial variety Kabulangeti (Holm-Sidak test, $P = 0.006$). Among mutation derived genotypes, none of the mutants differed significantly from the parent (CA) (Holm-Sidak test, $P > 0.05$). Error

bars represent standard error of the mean (SE). Common bean breeding lines and corresponding mutation derived genotypes were analysed separately. Genotype name; ADP 1 = Rozi Koko, ADP 466 = Mwezi Moja, ADP 684 = Majesty, ADP 765 = KK25

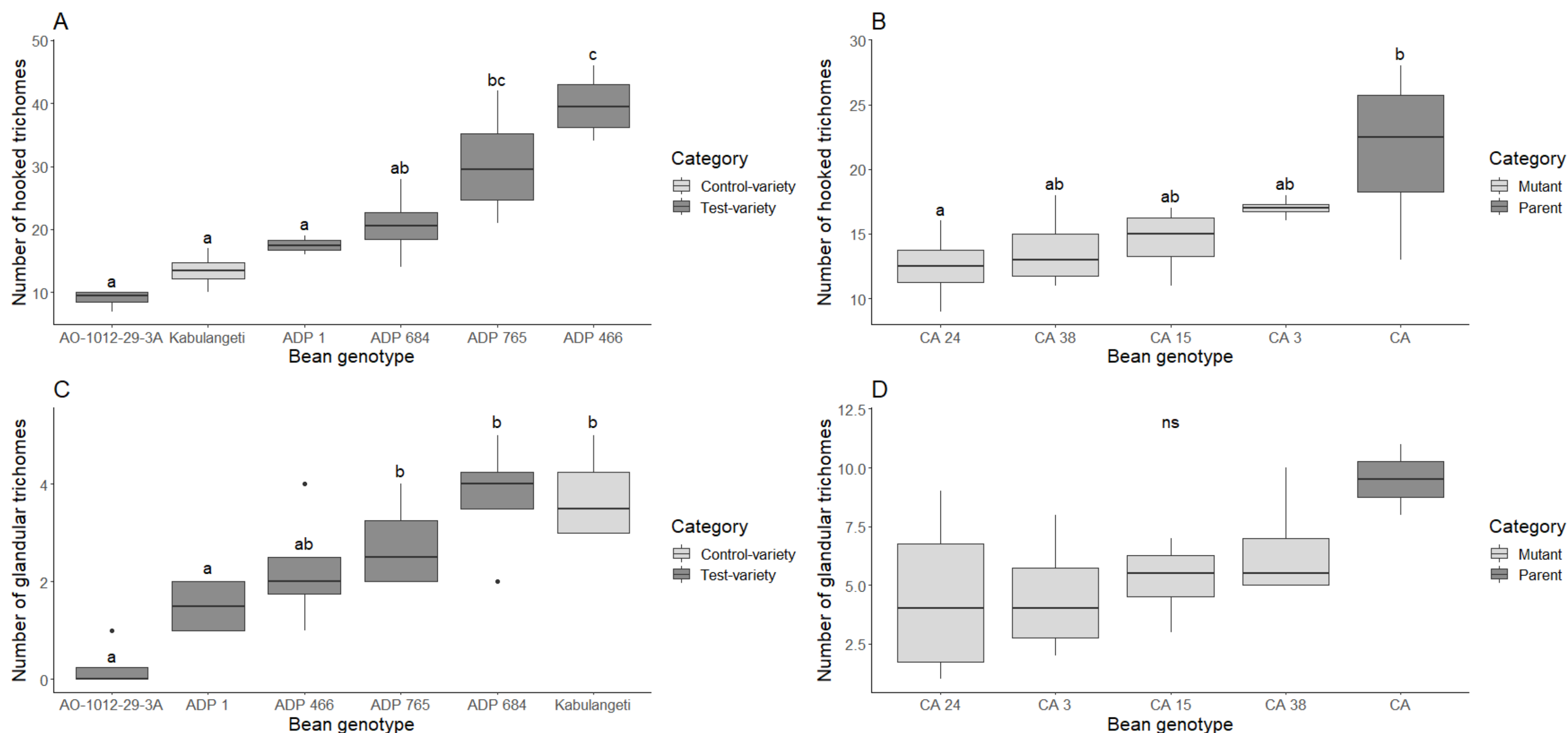

FIGURE 2 Median densities and confidence intervals of (A) hooked trichomes on common bean breeding lines (B) hooked trichomes on mutation derived genotypes (C) glandular trichomes on common bean breeding lines and (D) glandular trichomes on mutation derived genotypes, on lower leaf surfaces (n = 4). Among common bean breeding lines, ADP 765 (Holm-Sidak test, $P = 0.002$) as well as ADP 466 (Holm-Sidak test, $P < 0.0001$) had significantly higher numbers of hooked trichomes compared to the commercial variety.

For mutation derived genotypes, CA 24 had smaller numbers of hooked trichomes compared to the parent (Holm-Sidak test, $P = 0.03$). For glandular trichomes on common bean breeding lines, AO-1012-29-3A (Holm-Sidak test, $P = 0.001$) and ADP 1 (Holm-Sidak test, $P = 0.049$) had fewer trichomes compared to the commercial variety Kabulangeti. Common bean breeding lines and corresponding mutation derived genotypes were analysed separately. Groups followed by different letters are significantly different (Holm-Sidak post-hoc test), ns = non-significant differences between groups. Genotype name; ADP 1 = Rozi Koko, ADP 466 = Mwezi Moja, ADP 684 = Majesty, ADP 765 = KK25

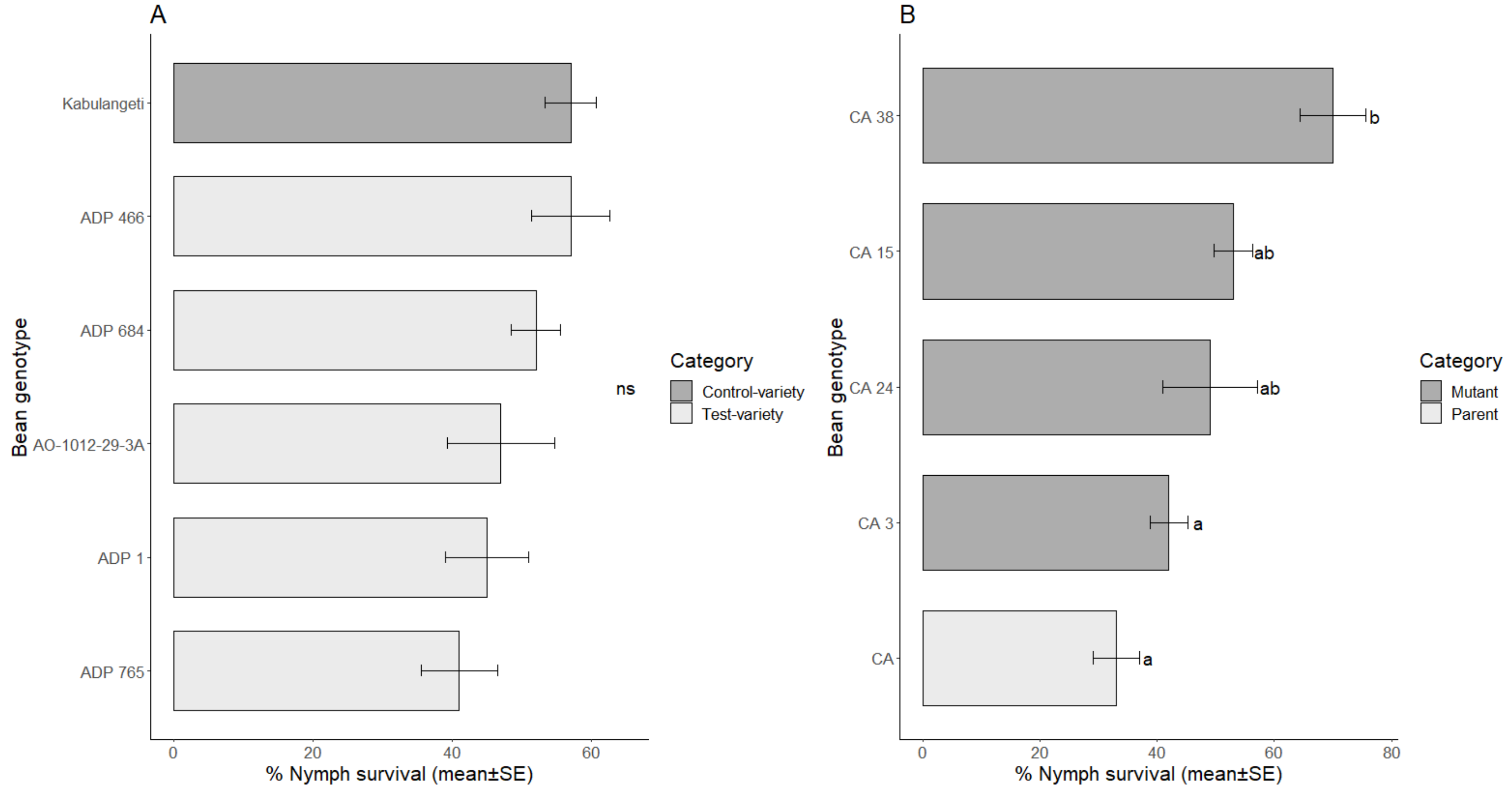

FIGURE 3 Black bean aphid nymph survival on (A) common bean breeding lines and (B) mutation derived genotypes (n = 10). Among the mutation derived genotypes, more aphids survived on CA 38 compared to the parent (CA) (Holm-Sidak test, P = 0.0001). Bars followed by different letters are significantly different (Holm-Sidak post-hoc test). ns = non-significant differences among bars. Error bars represent standard error of the mean (SE). Common bean varieties and corresponding mutation derived genotypes were analysed separately. Genotype name; ADP 1 = Rozi Koko, ADP 466 = Mwezi Moja, ADP 684 = Majesty, ADP 765 = KK25

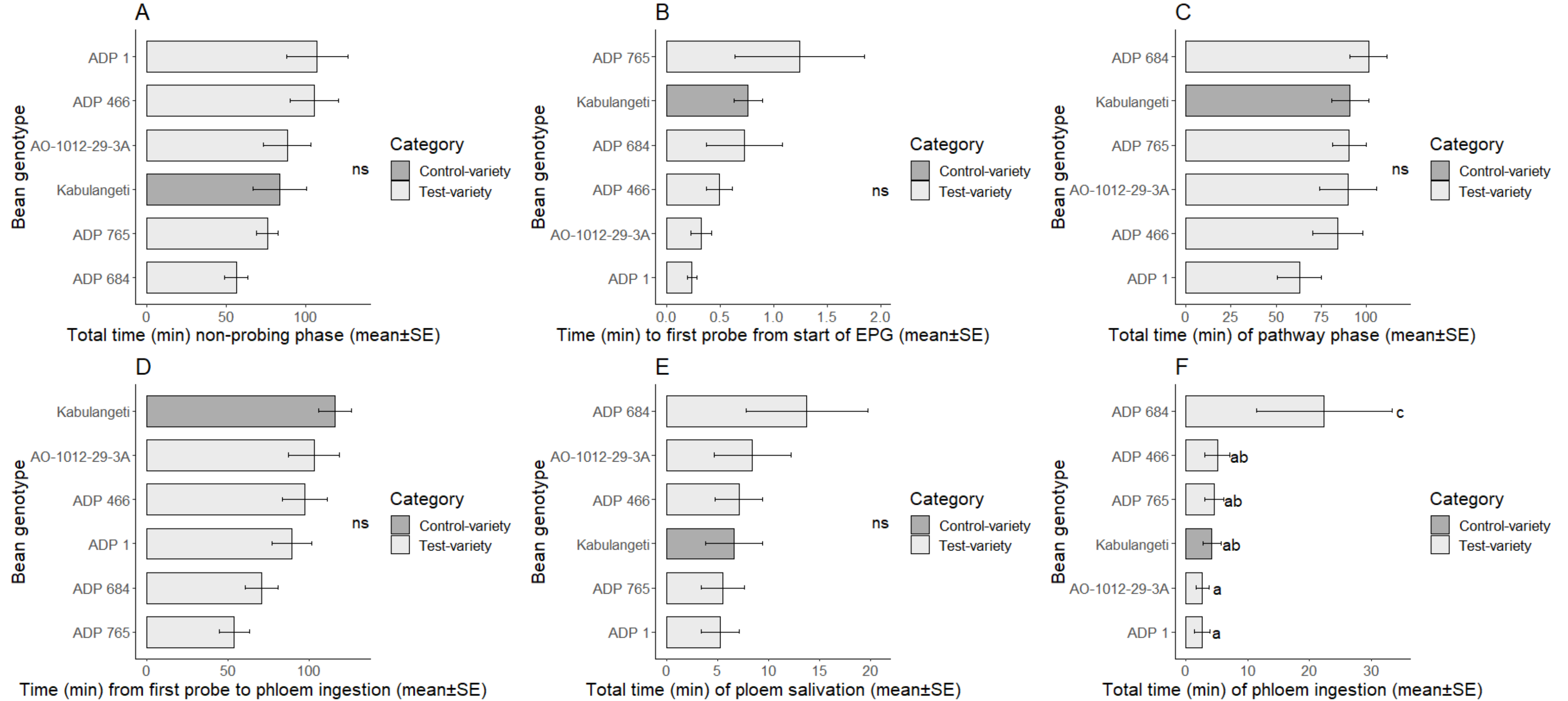

FIGURE 4 Aphid probing and feeding behaviour on common bean breeding lines. (A) total time of non-probing phase, (B) time to first probe from beginning of EPG recording, (C) total time of pathway phase, (D) time from first probe to sustained phloem ingestion, (E) total time of phloem salivation and, (F) total time of phloem ingestion (n = 14). In the case of phloem ingestion (4F), aphids fed longer on ADP 684 compared to the commercial variety Kabulangeti (Holm-Sidak test, P = 0.01), Common bean genotypes in figures A-F were analysed separately. 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. Genotype name; ADP 1 = Rozi Koko, ADP 466 = Mwezi Moja, ADP 684 = Majesty, ADP 765 = KK25