The University of Zambia

School of Agricultural Sciences
Department of Plant Science

COWPEA SEED PRODUCTION AND COMMERCIALIZATION

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8th JANUARY, 2024

1. Introduction

Cowpea is a major legume crop adapted to the semi-arid regions of the tropics with an average annual temperature of 30ºC and rainfall ranging between 800 mm to over 1,000 mm annually where other food crops do not perform well. It is a source of highly nutritious and inexpensive dietary protein and micronutrients for poorer households. The crop can provide fodder for improved livestock production and grain for human consumption. Cowpea has high nitrogen fixing capacity, has the ability to improve soil fertility, and is ideal to be grown in rotation with cereal crops.

2. Development of improved legume crop varieties

The Department of Plant Science in the School of Agricultural Sciences of the University of Zambia in collaboration with Zambia Agricultural Research Institute (ZARI), National Institute for Scientific and Industrial Research (NISIR), Enhanced Small Livestock Improvement Programme (E-SLIP) of the Ministry of Fisheries and Livestock and the International Atomic Energy Agency (IAEA) have developed improved legume cowpea varieties of Lukusuzi and Lunkhwakwa through Induced mutation breeding. The parental varieties of Lutembwe and Bubebe cowpea varieties were irradiated at NISIR Headquarters in Zambia. Following mutagenic treatment, selection for desirable traits using the Pedigree Method was carried out by the Department of Plant Science, UNZA through to M_6. The Department of Agricultural Economics of UNZA carried out Profitability and Technical Efficiency tests on the mutation-derived cowpea lines. Multi-locational and awareness trials were carried out by ZARI from M_6 until submission for release and commercialization in 1918. UNZA, IAEA largely funded the research. E-SLIP was mostly responsible for awareness and seed multiplication by cowpea seed growers.

3.0 Cowpea seed production

3.1 Basic seed production

There has been tremendous progress in seed production of Lukuzi and Lunkhwakwa cowpea varieties. In the 2022/23 growing season, about 3,000 kg of basic seed of Lukusuzi and Lunkhwakwa was produced by Pache-Pache seed growers based in Kabwe district of Zambia. The seed growers were sponsored by **E-SLIP** and overseen by E-SLIP and the Department of Plant Science of UNZA.

3.2 Procurement of Basic seed

3.2.1 In the follow up activities, arrangements were made in 2023/24 growing season for the procurement of the same Lukusuzi basic seed by 6 institutions for their seed growers based in Agro-ecological Regions III, IIA and IIB as presented in Tables 1and 2. The number of seed growers from the various institutions growing Lukusuzi basic seed   186 farmers

3.2.2 The amount of seed distributed to each farmer varied from 5 kg for farmer Dyness Chose Kalima of Luanshya on the Copper belt to 150 kg for farmer Frank Belly Mweene of Itezhi-tezhi in Central province. The 182 farmers included forage seed growers supported by E-SLIP who retained Lukusuzi seed for further multiplication (Table 2). The E-SLIP supported farmers included one farmer Kelvin Muyoyete Songiso from Nkeyema district of Western Province in Region IIB. Cassia Agro-Enterprise distributed Lukusuzi cowpea seed to Mwanachingwala Seed Growers Association, Hachanga Women's Club in Moonze, Siasikabole farmers in Choma and individual farmers. Cassia Agro-Enterprise itself obtained Lukusuzi cowpea seed.

Table 1: Procurement of Lukusuzi basic seed by institutions

Institution	Funding Agency	Amount of seed (kg)	Number of farmers	Seed distributed per farmer/institution (kg)	Locality of seed production
1. Self Help Africa	Self Help Africa	280	56	5	Chikankata, Kalomo (Southern Province)
2. Kagezi Seed	Kagezi Seed sponsored	30	6	5	Sinda (Eastern province)
3. Smaltech Laboratories Limited	GIZ	100	20	5	Kabwe (Central province), Lusaka (Lusaka province), Choma (Southern province)
4. E-SLIP	GRZ	665	19	5-150	As in Table 2
5. Cassia Agro Enterprise LTD	Cassia Agro Enterprise LTD sponsored	400	84	200 50 100 30 20	Mwanachingwalq Hachanga, Moonze Siasikabole, Choma Individual farmers Cassia Agro
6. Other	GRZ	150	1	150	Not provided
Total		1,625	186		

Table 2: E-SLIP Forage Seed Growers who retained Lukusuzi seed for further multiplication

ESLIP FORAGE SEED GROWERS						
S/N	**Full Name**	**Phone number**	**Alternative Phone number**	**District**	**Province**	**Quantity**
1	Doris Mulinyanga	0962514692	-	Kapiri Mposhi	Central	25
2	Dyness Chose Kalima	0960717342	-	Luanshya	Copperbelt	5
3	Elvis Mazuba	0968185131	-	Kapiri Mposhi	Central	25
4	Esther Ngambi	0979875295	-	Kabwe	Central	40
5	Evaness Kapemba	0953908689	0979211777	Kapiri Mposhi	Central	55
6	Felisters Nyendwa	0963401070	-	Kapiri Mposhi	Central	25
7	Frankbelly Mweene	0978348039	-	Itezhi-tezi	Central	150
8	Germany Lungu	0977134446	-	Lusaka	Lusaka	20
9	Getrude Chomba	0974407016	-	Kapiri Mposhi	Central	45
10	Grace Nachibanga	0973616772	-	Kapiri Mposhi	Central	25
11	Grace Phiri	0964237246	-	Chipata	Eastern	30
12	Joyce Kalaka	0963111543	-	Kapiri Mposhi	Central	25
13	Kelvin Muyoyete Songiso	0968676377	-	Nkeyema	Western	20
14	Kenny Katawa	0974855926	-	Chipata	Eastern	25
15	Margarate Chikoti	0966809892	-	Luanshya	Copperbelt	20
16	Marvis Kupa Dabula	0978966884	-	Kabwe	Central	50
17	Mary Chimboyi	0956146396	-	Kabwe	Central	25
18	Royce Lutabika	0957852610	-	Kabwe	Central	25
19	Saidon Zimba	0978231560	-	Lundazi	Eastern	20
	TOTAL					**655**

3.2.3 The total amount of basic seed procured for seed growers currently amounts to 1,625 kg. With a seed rate of about 15 kg/ha, the total cowpea hectarage expected to be covered is 108.3 ha. At a conservative cowpea grain yield of 2 ton/ha, this is expected to produce about 216.7 ton of Lukusuzi cowpea seed. This amount of seed is expected to increase further as other seed growers within the same E-SLP Forage seed growers group are still accessing the same Lukusuzi seed. The list will be updated by end of January to give a consolidated tonnage of Lukusuzi for seed production.

3.3 Branding of Lukusuzi cowpea seed

For Institutions requiring branding of seed packages, a sticker was developed through Smaltech Laboratories Limited. The local partners on the sticker are represented by Zambia Agriculture Research Institute (ZARI), National Institute for Scientific and Industrial Research (NISIR) and E-SLIP. The external partner is represented by the International Atomic Energy Agency (IAEA). A sample of the sticker is shown in Figure 1

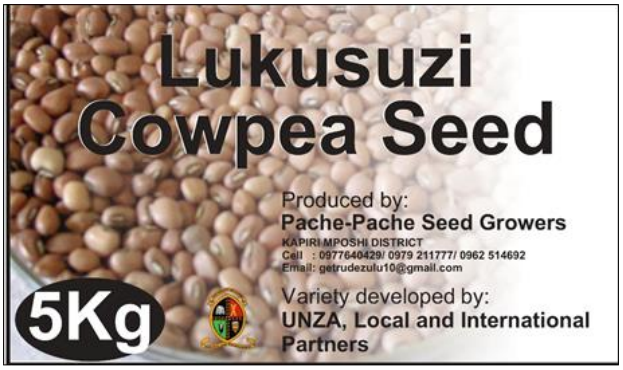

Figure 1: Sticker of Lukusuzi on the cowpea 5kg seed package

3.4 Category of seed

The seed growers are expected to grow Class 1 Lukusuzi seed. This should mark the first start of commercial production of the mutation-derived Legume of Lukusuzi cowpea variety and possibly Lunkhwakwa.

3.5 High performing variety and good quality seed

3.5.1 **L**utembwe cowpea variety produced by Seed Companies has been on the market since **1988 and is now an admixture of Lutembwe and Bubebe and should be replaced (Figure 2).**

Figure 2: Bubebe variety currently grown and displayed at the National Pasture and Livestock Fair held at GART- Batoka in November, 2023 was a phenotypical admixture of Lutembwe and Bubebe cowpea varieties.

Lutembwe can be replaced by Lukusuzi which is a mutation-derived variety from the Lutembwe parental variety. It has similar characteristics to Lutembwe but with superior characteristics than the parental variety as follows:

3.5.2 Higher grain yield of more than 25% compared to its parental variety Lutembwe with expected increase in farmers' income of more than 25% or by an extra 150 US$/ha,

3.5.3 High tolerance to soil acidity and Aluminium toxicity resulting in higher biomass (Figure 3A) and grain yield (Figure 3 B and Figure 5) in Regions with higher rainfall and with predominantly acidic soils

3.5.4 More tolerant to diseases such as *Ascochyta* and *Cercospora* (Figure 3A) compared to the Lutembwe parental variety which is susceptible to fungal diseases resulting in very low grain yields (Figure 4)

Figure 3: High Lukusuzi biomass (A) and grain yield (B) were recorded in Kalulushi district of the Copperbelt province in Region III despite the high rainfall with predominantly acidic soils and high Aluminium toxicity and prevalence of fungal diseases. The farmer in picture A did not spray with any fungicides for control of fungal diseases, confirming tolerance of the variety to fungal diseases.

Figure 4: Severe incidence of fungal diseases on Lutembwe parental variety at Expo 2022 at GART in 2022. Lutembwe parental variety is severely affected by fungal diseases greatly reducing the biomass and resulting in very low grain yields.

3.5.5 More drought tolerant than Lutembwe. The plasticity of the variety allows for better partitioning of plant biomass to pods (Figure 6), with reduced rainfall.

3.5.6 Lukusuzi is to some measure also dual purpose, with grain for human consumption and fodder for improved livestock production (Figure 6).

Figure 5: High grain yield of Lukusuzi in storage at Kalulushi on the Copperbelt in the 2022/23 growing season. Note as in Figure 3 (B), Lukusuzi has characteristically long pods, resulting in higher number of seeds per pod and therefore the resulting high grain yield. Insert: Good quality harvested Lukusuzi cowpea seed

4. Increase in cowpea production in Zambia

With use of Lukusuzi and Lunkhwakwa, mutation-derived varieties from Lutembwe and Bubebe parental varieties, production of cowpea is, therefore, expected to increase in Provinces with higher rainfall but currently with much lower cowpea production such as North-Western, Northern, Muchinga, Luapula and Copperbelt provinces. This is because of predominantly acidic soils and aluminium toxicity in these soils as well as high prevalence of fungal diseases. This should lead to total increase in national cowpea production.

Figure 6: Tolerance of Lukuzi to adverse environmental conditions allows for better partitioning of plant biomass to pods, with reduced

5.0 Potential to replace Lutembwe

5.1 Apart from replacement of Lutembwe with Lukusuzi, replacement of Lutembwe could also include LT4-2-4-1 and LT11-3-3-12 but also LT-3-8-4-1 and LT3-8-4-6. The grain yield of LT4-2-4-1 was 2.3 and 2.1 times more than that of the parental variety at UNZA in Region IIA with medium rainfall (800 -1000 mm) and at Chirundu (CHRD) in Region I respectively with lower rainfall (< 800 mm). Similarly the grain yield of LT11-3-3-12 was 1.6 and 1.8 times more than that of the parental variety at UNZA and Chirundu respectively (Figure 7).

5.2 LT4-2-4-1 is one of the highest yielding cowpea mutation derived line developed across Agro-ecological Regions.

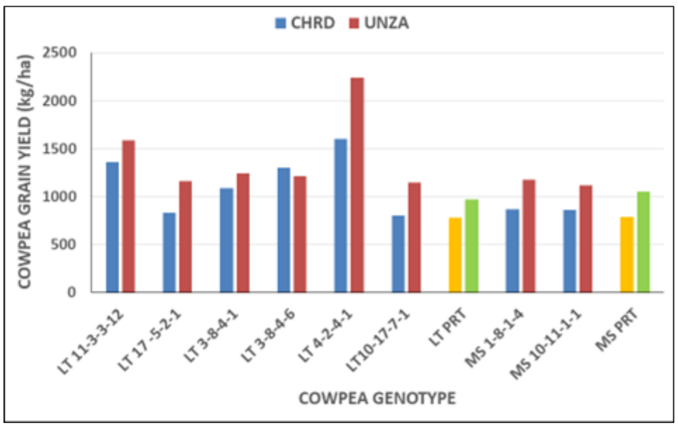

Figure 7: Variation of grain yield with site and Cowpea genotype in the 2022/23 growing season

The mutant is characterized by having more pods per peduncle (2,3) than the parental variety, further more increasing the grain yield. Synchronous in podding characteristic of LT4-2-4-1 allows for commercial production and combine harvesting.

5.3 LT11-3-3-12 is also high yielding in both biomass and grain yield. It has stay green characteristic making it dual purpose with grain for human consumption and fodder for livestock production. It is highly drought tolerant and one of the most tolerant mutant to soil acidity and Al toxicity. LT11-3-3-12 has high biological nitrogen fixing capacity (determined through N-15 Dilution Technique) compared to the parental variety providing high residual soil nitrogen for cereal crops in rotation such as Maize or Sorghum. The mutant can be used as an intercrop with cereals as it shades weeds and **increases soil moisture** content in the succeeding cereal crop in rotation, LT11-3-3-12 provides high grain yields under **low phosphate levels and moisture stress**.

5.4 The high grain yield potential of LT4-2-4-1 and LT11-3-3-12 can also be attributed to resistance to aphids. The two mutants are resistant to aphids characterized by lower aphid colony growth, mean relative growth rate, intrinsic rate of natural increase and, population doubling time compared to the parental variety (Zimba *et al*., 2022).

5.5 Although Lutembwe is susceptible to aphids, a study by Zimba *et al*., (2023), showed that aphid resistance of this variety may be enhanced with application of silicon. Applying silicon to LT4-2-4-1 and LT11-3-3-12 increases the level of silicon in the plants but does not result in enhanced resistance to aphids. Alternatively, it is possible that the lower aphid performance on mutation-derived genotypes may have reduced the apparency of additional benefit of silicon application.

5.6 Silicon application or use of mutation-derived genotypes may be effective tools with which to manage cowpea aphid on cowpea, but there appears to be little benefit of combining these approaches. These findings have important implications for developing an integrated pest management framework for aphid pests on cowpea.

5.7 The high yield potential and tolerance to adverse environmental conditions led the Department of Plant Science to propose LT4-2-4-1 and LT11-3-3-12 for fast track testing and release so that the two mutants are available to farmers sooner!

6.0 Cross resistance to pests and pathogens

6.1 While BB8-1-7-5 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 *et al.*, 2022c).

6.2 *The Ra* gene, for example, 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 *et al.*, 2022c). 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 characterization of genotypes BB7-9-7-5 LT 4-2-4-2 and LT 11-3-3-12 requires further work to elucidate mechanisms of resistance.

7.0 Evaluation of cowpea aphid performance on silicon treated cowpea genotypes under laboratory and field conditions

7.1 An important challenge with aphid management using resistant cultivars is the ability of aphids to develop new virulent biotypes that overcome host-plant resistance (Botha, 2021). It is well established that deploying multiple control measures such as the use of resistant varieties, biocontrol agents and insecticides within an IPM framework reduces the likelihood of aphid species evolving virulent biotypes (Jaouannet *et al.*, 2014; Botha, 2021). In order to prepare for the potential breakdown of aphid resistance in selected mutant cowpea genotypes, a study was conducted to investigate if silicon application improves aphid resistance. Results of the study indicated that silicon application did not complement mutation-derived aphid resistance in LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12 genotypes, likely due to the lack of interaction between silicon and aphid resistance mechanisms in these genotypes. However, silicon application improved aphid resistance in Lutembwe parent genotype.

7.2 Reduced aphid performance on Lutembwe genotype suggests that silicon application may be a promising approach for managing cowpea aphid on this genotype, and could complement existing aphid management efforts in cowpea varieties.

8.0 Lunkhwakwa basic seed production

8.1 There has been little progress in **Lunkhwakwa basic seed production.** It appears farmers did not appreciate Lunkhwkwa mutation-derived cowpea variety. Lunkhwakwa actually performs better than the parental variety Bubebe. The variety is one of the most tolerant to soil acidity and Aluminium toxicity. The grain yield of Lunkhwakwa can be as much as 4 ton/ha because of the high number of pods per plant compared to Bubebe parental variety where the yield can be less than a ton/ha. In addition, Lunkhwakwa has stay-green characteristic being dual purpose with grain for human consumption and fodder for improved livestock production.

8.2 The variety requires greater awareness of its good attributes compared to the Bubebe parental variety. Field observations under rainfall conditions have also shown **Lunkhwakwa to be likely to be tolerant to aphids.** The study of Siyunda found Lunkhwakwa to be resistant to aphids (Siyunda, 2022b).

9.0 Collaboration with E-SLIP

9.1 E-SLIP has played a pivotal role in the promotion of mutation-derived and improved cowpea varieties of Lukusuzi and Lunkhwakwa as well as other mutation-derived cowpea lines with high yield potential and with desirable traits such as LT4-2-4-1 and LT11-3-3-12. This is described in the report on "Improvement and development of improved cowpea varieties Lukusuzi and Lunkhwakwa" submitted.

9.2 The E-SLIP has carried out nutritional studies on the cowpea and velvet bean mutants provided by the UNZA Legume Improvement programme. It has carried studies on intercropping of cowpea and Velvet bean mutants with cereals and subsequent feeding the intercrops to livestock.

9.3 The E-SLIP displayed basic seed of Lukusuzi at the Agricultural and Commercial Show in August 2023 (Figure 8).

Figure 8: Display of Lukusuzi improved cowpea variety by Mr. German Lungu, Chairperson of the cowpea Growers Association supported by E-SLIP at the Agricultural and Commercial Show in Lusaka

9.4 E-SLIP also displayed good quality basic seed of Lukusuzi at the National Pasture and Livestock Fair held at GART- Batoka in November, 2023 (Figure 9).

Figure 9: Display of Lukusuzi cowpea seed at the National Pasture and Livestock Fair held at GART- Batoka in November 2023. In the background is the display of mutation-derived cowpea lines LT4-2-4-1, LT11-3-3-12, LT3-8-4-1 and LT3-8-4-6 as well as Velvet bean mutant lines in the pipeline for release and commercialization.

9.5 Good quality Lukusuzi cowpea basic seed was produced by Pache - Pache Seed Growers of Kabwe district. The cowpea Seed growers were supported and overseen by E-SLIP and the Department of Plant Science, UNZA. Lukusuzi basic seed produced was branded in 5 kg (Figure 1) and 25 kg bags (Figure 10). The seed was evaluated and determined to be good quality Lukusuzi basic seed (Figures 11 and 12).

Figure 10: Lukusuzi cowpea variety in 25 kg branded bag

Figure 11: UNZA technicians Ms. Sharon Matenga and Mr. Tobias Alubi with the Chairman (middle) of the Cowpea Seed Gowers Association found Lukusuzi cowpea variety to be good quality basic seed

Figure 12: Good quality basic seed of Lukusuzi Cowpea variety

10.0 Research still required

10.1 Fast tracking and release of LT 11-3-3-12 and LT 4-2-4-1

Following the evaluation and determination of the desirable traits of LT4-2-4-1 and LT11-3-3-12, the Department of Plant Science proposed fast track seed testing and release of these cowpea lines during the 2021/22 growing season so that they could be available to farmers sooner. The attributes of the mutants are described in the attached document on "Improvement and Development of Improved cowpea Varieties of Lukusuzi and Lunkhwakwa." Unfortunately, this was not implemented due to lack of funds. Fast tracking, however, attracts extra resources that are supposed to be paid by the client. The cost will include; land hire, weeding, employing trial assistants, fertilizers and any other incidental costs. Costs for managing extra sites for fast tracking amount to K500, 000 (US$ 18,500.00 The figure may vary depending on the site, distance etc. The Grand Total amounts to USD 2,500 + USD18, 500.00 = USD 21,000

10.2 Conventional testing and release

The Department of Plant Science is considering conventional testing and release of the following mutation-derived cowpea lines:

(i) LT3-8-4-1, (ii) LT3-8-4-6, (iii) BB14-16-2-2, (iv) BB10-4-2-3, (v) LT11-5-2-2, (vi) MS10-11-1-1.

The normal seed release process consists of:

Official Variety Release Trials,

(1) Registration fee: USD 20 x 4varieties =USD 80,
(2) DUS testing fee: USD150 x 4 varieties x 1site x 2years = USD 1,200:
(3) VCU testing fee: USD150 x 4 varieties x 6 sites x 2years = USD 7,200
(4) Variety Release Fee: USD 30 x 4 varieties =USD 120

The cost for the release of one cowpea line is USD 2,150. The total cost for release of 5 cowpea lines amounts to US$ 8,600.00.

10.3 Multiple Location trials

The Department of Plant Science is proposisng to conduct multiple location trials at the following location; Mansa, Msekera, Lusaka, Mochipapa, Simulumbe and Lusitu. The following mutation-derived cowpea lines will be evaluated.

Table 3: Mutation-derived cowpea lines for multiple location studies

Number	Cowpea mutant line	Number	Cowpea mutant line
1.	Lukusuzi	11.	LT17-5-2-1
2.	Lunkhwakwa	12.	BBT-11
3.	LT3-8-4-1	13.	BBT-1-7,
4.	LT3-8-4-6	14.	BB3-9-7-5
5.	BB14-16-2-2,	15.	BB7-9-7-5
6.	BB10-4-2-3	16.	BB8-9-7-5
7.	LT11-5-1-1	17.	MS1-8-2-6-8-1
8.	LT11-5-2-2-2	18.	MS1-8-4-1
9.	LT10-7-1-12	19.	MS1-8-1-4
10	LT17-5-2-1	20.	MS10-11-1-1

10.4 Seed Multiplication

Seed multiplication is proposed for the following cowpea lines presented in Table 4

This will be carried out locally at the Field Research Station or at the University of Zambia and University Liempe farm. This requires a total of K-- to implement this activity.

Commented [KZ1]: Amount to be included

Table 4: Mutation-derived cowpea lines for seed increase

Number	Cowpea mutant line	Number	Cowpea mutant line
1.	LT-4-2-4-1		BB10-4-2-3
2.	LT11-3-3-12		BBT1-11
3.	LT3-8-4-1		BBT-1-7,
4.	LT3-8-4-6		BB3-9-7-5
5	LT11-5-1-1		BB7-9-7-5
6	LT11-5-2-2		BB8-9-7-5
7	LT11-5-2-2-2		MS1-8-2-6-8-1
8	LT10-7-1-12		MS1-8-4-1
9	LT17-5-2-1		MS1-8-1-4
10	BB14-16-2-2		

10.5 Nitrogen fixing capacity in legumes

10.5.1 We are planning to carry out 2 studies on nitrogen fixing capacity in legumes one on common bean and the other on cowpea. More than 100 mutation-derived cowpea lines have been developed by the Department of Plant Science through induced mutation. There is need to evaluate the nitrogen fixing capacity of cowpea mutants identified with high yield potential and with tolerance to pests and diseases.

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11.0 Cross resistance

Of particular interest should be paid to cowpea lines found to have cross resistance to both aphid and Bruchid (Zimba *et al*., 2022). This should also include mutant lines which were identified to possess different resistance traits, for example, MS1-8-2-6-8-1 (aphid resistance only), LT11-5-2-2-2 (bruchid resistance only), and BBBT1-11 (aphid and bruchid resistance). Phenolics were identified to be highly responsible for the multiple resistant trait expressed by BBBT1-11 (Siyunda et al., 2022b). Resistance is possibly under the control of three or more genes. BBBT1-11 can be utilized in breeding programs with a target of breeding multiple insect resistant cowpea genotypes. Further research should also be conducted in order to genotype the BBBT1-11 and identify the QTLs responsible for the trait observed in BBBT1-11. Aphid and pathogen resistant gene are often clustered on the same region of the chromosome. The same common genomic locale of aphid and pathogen resistance genes, may have induced genetic variations for both pathogen and aphid resistance traits. Apart from having cross resistance to aphid and Bruchids, Lukusuzi and Lunkhwakwa and other selected mutants appear to have also cross resistance to Al toxicity. These cowpea lines, therefore, require further evaluation.

12.0 Biological nitrogen fixation (BNF)

12.1 More than 100 common bean and cowpea lines have been developed through crossing and induced mutation breeding. As with cowpea mutants, common bean lines with high yield potential and with tolerance to pests and diseases as well as vastly reduced cooking time should be evaluated for nitrogen fixation.

12.2 BNF is best evaluated with the use of the N-15 Dilution Technique. The extent to which the $^{15}N/^{14}N$ ratio in the fixing crop is decreased, relative to the non-fixing plant provides a direct and quantitative measure of the N_2 fixing ability, and can be used to estimate N_2 fixed in the field.

12.3 The amount of N-15 labelled urea at 5 Atom % N-15 required for evaluation of BNF in cowpea is 2 kg, Similarly, the amount of N-15 labelled urea at 5 Atom % N-15 required for evaluation of BNF in common is 2 kg,

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