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Inheritance and Allelic Relationships of *Alectra vogelii* Benth. Resistance Genes in Cowpea Genotypes B301 and KVx414-22-2

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Abstract

Alectra vogelii Benth. is the second most important parasitic weed in cowpea [*Vigna unguiculata* (L.) Walp.] production in Burkina Faso. Several resistant varieties to this weed have been identified in the country among which are B301 and KVx414-22-2. The inheritance and allelic relationships of the resistance genes in the two varieties have not been studied with *A. vogelii* strains in Burkina Faso. The objective of this study was to determine the inheritance and allelic relationship of the resistance genes in B301 and KVx414-22-2. To determine the inheritance of the genes for resistance, the resistant varieties (B301 and KVx414-22-2) were each crossed to a susceptible variety IT82D-849 to generate F_1 and F_2 populations. For the allelic relationship study the two resistant genotypes were crossed among themselves to generate F_1 and F_2 offspring. The parents and their F_1 and F_2 progenies were screened in artificially infested pots with *Alectra* seed in a screen house at Kamboinsé Research Station in Burkina Faso. Resistance/susceptibility of genotypes was assessed by recording the number of emerged *Alectra* shoots. The data were subjected to the Chi-Square goodness-of-fit test for one, two and three genes segregation ratios. The results revealed that two independent dominant genes confer resistance in the variety B301 and a single dominant gene confers resistance in variety KVx414-22-2. The single dominant gene in KVx414-22-2 is non-allelic to the two genes in B301. The two resistance genes in variety B301 have already been named *Rav1* and *Rav2* whilst *Rav3* is the name of the resistance gene in variety IT81D-994. Therefore, we propose the symbol *Rav4* as the name for the resistance gene in variety KVx414-22-2.

Keywords: *Alectra vogelii*, allelic relationship, dominance, resistance gene, *Vigna unguiculata*

1. Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is one of the major grain legumes produced in semi-arid and arid areas of the world. The high protein quality of this legume for both human and animal nutrition is acknowledged worldwide. Its production also generates cash income for stakeholders.

Africa is by far the largest cowpea producing continent in the world. However, the African continent is also the area where the most devastating cowpea production constraints prevail (drought, insect pests, diseases and parasitic weeds) (Singh & Allen, 1979; Horn & Shimelis, 2020). Both abiotic and biotic constraints very often lead to significant yield losses. Parasitic weeds *Alectra vogelii* and *Striga gesnerioides* are two major cowpea production constraints in Africa. *Alectra vogelii*, though less widely studied than *Striga gesnerioides*, is widespread and causes severe damage to cowpea production (Mohamed et al., 2006; Kabambe, Tembo, & Kazira, 2013). Yield losses ranging from 80% to 100% due to *A. vogelii* infestation have been reported (Mbwaga, Hella, Mligo, Kabambe, & Bokosi, 2011; Kabambe et al., 2013).

Several control measures (cultural practices and chemical control) have been used with ineffective results because of their cost, technical difficulties and inaccessibility. Therefore, genetic resistance remains the most efficient, affordable and environmentally friendly method for controlling this weed (Rubiales et al., 2006). Varieties conferring resistance to *A. vogelii* have been identified. However, the inheritance of the resistance in most of these varieties is yet to be investigated. (Atokple, Singh, & Emechebe, 1993) and (Singh et al., 1993) reported two independent dominant genes, namely $R_{av}1$ and $R_{av}2$ in landrace B301 from Botswana. However,

this variety does not have farmers' preferred traits (large seed size, rough and white seed) for the Burkina Faso market. It has small, smooth, brown seeds controlled by alleles that are dominant over those for large white seed (Noubissié et al., 2011). Therefore, using B301 as a donor parent for *Alectra* resistance while improving for farmers' preferences will slow down the selection process. Nevertheless, it remains a good donor parent because it combines resistance to both *Alectra* and *Striga*. It was also reported that a single dominant gene which was given the symbol, *Rav3*, confers resistance to *Alectra* in the variety IT81D-994 (Atokple, Singh, & Emechebe, 1995; Kouakou et al., 2009) which has farmers' preferred traits but this gene does not confer full resistance to *Alectra* (Atokple et al., 1995; Kouakou et al., 2009; Dieni et al., 2018). New sources of resistance to the weed, including B301 and KVx414-22-2, have been identified in Burkina Faso through both screen house and field screenings (Dieni et al., 2018). In addition to *Alectra* resistance, the variety KVx414-22-2 possesses some farmers' accepted traits. However, the inheritance patterns of the resistance gene(s) in this variety and the allelic relationships with those in variety B301 have not been studied. The objectives of this study were to (i) determine the inheritance patterns of the resistance in the varieties B301 and KVx414-22-2, and (ii) determine the allelic relationships between the genes conferring resistance to *Alectra vogelii* in these varieties.

2. Materials and Methods

2.1 Population Development

The genetic material used in this study comprised a susceptible genotype, IT82D-849 and two resistant genotypes, B301 and KVx414-22-2 (Dieni et al., 2018) used as parents. These parents were used in two sets of crosses. On one hand, the susceptible parent was crossed with each of the resistant parents to produce F_1 families (F_1 IT82D-849/B301 and IT82D-849/KVx414-22-2). The F_1 plants were self-pollinated to generate F_2 progenies (F_2 IT82D-849/B301 and IT82D-849/KVx414-22-2). On the other hand, the resistant parents were crossed between themselves to develop F_1 (B301/KVx414-22-2) and F_2 offspring (B301/KVx414-22-2) through self-pollination of the F_1 individuals. Overall, three populations of F_1 and F_2 progenies were developed from the three crosses.

2.2 Experimental Management and Data Collection

The three parents IT82D-849, B301, KVx414-22-2 and their F_1 and F_2 offspring were used in this study. Ten (10) individuals of each parent as well as the F_1 populations were screened in a screen house at Kamboinsé Research Station for their reaction to *Alectra vogelii*. For each of the three F_2 populations two hundred (200) individuals were evaluated in the same experiment.

The experiment was conducted in screen house at Kamboinsé Research Station from June to August 2017. Plants were screened in plastic pots of ten (10) L filled with 12 kg of sterile soil. The pots were infested with *Alectra vogelii* seeds, collected from an *Alectra* infested field in Koupela (Burkina Faso) in October at the end of the 2014 rainy season. The seeds were dried under shade and sieved with a suitable (250-300 μm) mesh sieve. The sieved seeds were stored at room temperature until use. Each pot was infested with about 1,000 *Alectra vogelii* seeds based on the recommendations of Musselman and Ayensu (1983) and Magani et al. (2008). The preconditioning of the *Alectra vogelii* seeds consisted of watering the infested pots for two weeks in order to break seeds dormancy and ensure their uniform germination. This period of time has been reported to be optimal for preconditioning *Alectra* seeds (Magani et al., 2008). A single cowpea seed was planted per pot per genotype. Each parent and F_1 population was planted in ten pots; 200 pots were used for each of the F_2 populations making a total of 660 pots. The pots were kept moist by watering when it was necessary. From six (6) to ten (10) weeks after cowpea planting, pots were carefully checked to record the number of *Alectra vogelii* shoots that emerged. Plants which supported *Alectra* emergence were considered as susceptible; otherwise they were classified as resistant.

2.3 Data Analysis

The data collected were subjected to Chi-square "goodness of fit" test at 5% level of significance. For the inheritance studies, the segregation ratios were compared to Mendelian segregation ratios for one (3R:1S) and two genes (15R:1S). The hypothesis tested was that the observed segregation patterns follow Mendelian ratios. This hypothesis was rejected when the chi-square value was significant (the calculated chi-square was greater than the theoretical chi-square) otherwise it was accepted (the calculated chi-square was less than the theoretical chi-square).

To determine the allelic relationship between the genes for resistance in the two parents, the Mendelian's ratios for two dominant genes (15R:1S) and three dominant genes (63R:1S) were tested. The assumptions underlying this analysis are as follows:

(i) If two dominant genes A and B confer the resistance and each of the homozygous parents carry different resistance gene, then the parents can be AAbb and aaBB respectively. F_1 progenies derived from a cross between them will be AaBb which are resistant because of the presence of dominant A and B genes;

(ii) If the genes are allelic, then both the first (F_1) and the second (F_2) generations derived from a cross between these two resistant parents are expected to comprise only resistant progenies. In this case the parents would have been AA and AA respectively.

(iii) If they are not allelic segregation ratio in the F_2 generation will be consistent with the normal Mendelian ratio: 9A_B_:3A_bb:3aaB_:1aabb. All progenies carrying at least a dominant allele are expected to be resistant whilst susceptible progenies are those with homozygous recessive alleles (aabb) to give a ratio of 15R:1S.

(iv) If three dominant genes A, B and C confer the resistance and one of the homozygous parents carries two different resistance genes all different from the resistance gene in the second homozygous parent, then the parents can be AABBcc and aabbCC or other suitable combinations where one homozygous parent has two dominant genes at two loci but recessive on the third and the other homozygous parent has one dominant gene corresponding to the recessive locus in the in the first parent and recessive genes corresponding to the dominant loci of the other parent. A cross between them generates F_1 progenies of fully heterozygous genotype AaBbCc which are resistant because of the presence of dominant A, B and C genes.

(v) If the genes are allelic, then a cross between two resistant individuals would generate only resistant progenies in both the F_1 and F_2 generations. In this case the genotypes of the parents could be AABB and aaBB or AABB and AAbb.

(vi) If they are not allelic, segregation ratio in the F_2 generation will be in agreement with the normal Mendelian ratio: 27A_B_C_:9A_B_cc:9A_bb_C_:9aaB_C_:3A_bbcc:3aaB_cc:3aabbC_:1aabbcc. All progenies carrying at least a dominant allele are expected to be resistant whilst susceptible progenies are those with homozygote recessive alleles (aabbcc) to give a ratio of 63R:1S.

The equation for the Chi-square is as shown below:

$$\chi^2 = \sum_{i=1}^{k} (O_i - E_i)^2/E_i \quad (1)$$

Where, χ^2 = Chi-Square value; O_i = observed frequency of class i; E_i = expected frequency of class i; k = number of classes.

3. Results

3.1 Inheritance Patterns of the Resistance Genes

Results from the screening of the non-segregating populations are as follows: all the 10 individuals of the susceptible parent (IT82D-849) screened supported severe *Alectra* infestation while the resistant parents (B301 and KVx414-22-2) as well as both F_1 populations were free of *Alectra* infestation.

Among 200 F_2 progenies derived from a cross between IT82D-849 and B301, 18 individuals were susceptible (S) to *Alectra* while 182 were resistant (R). In the second F_2 population (IT82D-849/KVx414-22-2), 62 individuals out of 200 supported *Alectra* shoots emergence while 138 were resistant. The segregation ratios are presented in Table 1. The chi-square "goodness-of-fit" test for segregation patterns of the F_2 population from IT82D-849/B301 showed a good fit to a 15R:1S ratio (chi-square value 2.58, $P < 0.0.05$) with 182R:18S observed against expected values of 187.5R:12.5S. The segregation patterns in the F_2 population from IT82D-849/KVx414-22-2 was a good fit to a 3R:1S ratio (chi-square value 3.09, $P < 0.05$) with 138R:62S against expected values of 150R:50S (Table 1).

Table 1. Segregation patterns of the inheritance of *Alectra vogelii* resistance in two F_2 populations of cowpea

Cross	Total	Observed frequencies		Expected Frequencies		Ratio	χ^2	p-value
		R	S	R	S			
IT82D-849/ B301	200	182	18	187.5	12.5	15:1	2.58	**0.108**
IT82D-849/KVx414-22-2	200	138	62	150	50	3:1	3.09	**0.079**

Note. R: resistant, S: susceptible, χ^2 = Chi-Square value.

3.2 Allelic Relationship

The segregation patterns of F_2 progenies derived from the cross between the two resistant genotypes (B301/KVx414-22-2) are presented in Table 2. For the 200 F_2 plants tested only four were susceptible to *Alectra vogelii* and 196 were resistant. The χ^2 goodness-of-fit test was a good fit to a 63R:1S ratio (Chi-square value 1.63, $P < 0.05$) (Table 2). Therefore, the F_2 population segregated into a ratio 63 resistant: 1 susceptible.

Table 2. Segregation ratios of the allelic relationships of *Alectra vogelii* resistance in F_2 population derived from B301 and KVx414-22-2

Cross	Total	Observed frequencies		Expected frequencies		Ratio	χ^2	p-value
		R	S	R	S			
B301/KVx414-22-2	200	196	4	192.59	7.41	63:1	1.63	**0.202**

Note. R: resistant, S: susceptible, χ^2 = Chi-Square value.

4. Discussion

The resistant parents (B301 and KVx414-22-2) and all F_1 progenies derived from the different crosses were resistant to *Alectra vogelii*. The susceptible parent (IT82D-849) showed high degree of infestation confirming its susceptibility. The homogeneity of the F_1 progenies for resistance to *Alectra vogelii* demonstrated that at least one dominant gene is responsible for the resistance in each of the varieties. A dominant inheritance patterns was reported for B301 (Atokple et al., 1993; Singh et al., 1993).

The F_2 progenies derived from the cross between IT82D-849 and B301 segregated into a ratio of 15R:1S confirming that two dominant genes confer the resistance to *Alectra vogelii* in B301as reported by Atokple et al. (1993) and Singh et al. (1993). The F_2 offspring from IT82D-849/KVx414-22-2 assorted into a ratio of 3R:1S. The segregation ratio conforms with the Mendelian segregation ratio for one dominant gene conferring resistance to *A. vogelii*. Therefore, a single dominant gene governs the resistance in KVx414-22-2. Single dominant gene inheritance patterns was reported for *Alectra* resistance in IT81D-994 (Atokple et al., 1995; Kouakou et al., 2009). Several dominant genes (*Rsg1*, *Rsg2*, *Rsg3* and *994-Rsg*) have been reported for cowpea resistance to *Striga gesnerioides* in different genotypes including B301, IT82D-849 and IT81D-994; among them Rsg_1 and Rsg_2 have been shown to be allelic (Atokple et al., 1993, 1995; Ouedraogo et al., 2001; Singh et al., 1993; Tignegre, 2010).

For the allelic relationship study, the observed segregation ratio of the cross between the two resistant genotypes was similar to the inheritance patterns of a character governed by three independent dominant genes. The independent inheritance patterns of the two genes conferring resistance in B301 has been reported by Atokple et al. (1993) and confirmed by the present study. Thus, if one of these two genes was allelic to the resistance gene in KVx414-22-2, then the segregation ratio of the F_2 progenies derived from B301/KVx414-22-2 should be consistent with Mendelian's segregation patterns for two independent genes. However, this hypothesis was rejected ($p < 0.05$) because a ratio of 63 resistant to 1 susceptible indicated that the three genes were independent and located on three different chromosomes. Therefore, it could be concluded that the two genes conferring resistance to *Alectra* in B301 and the one responsible for resistance in KVx414-22-2 are not allelic. Non allelic relationship between the genes conferring resistance to *Alectra vogelii* in B301 and IT81D-994 has also been reported (Atokple et al., 1995). Therefore, the symbols Rav_1, Rav_2 and Rav_3 were proposed for the two resistance genes in B301 and the one gene in IT81D-994 respectively (Singh et al., 1993; Atokple et al., 1995). In contrast to the fully resistant varieties B301 and KVx414-22-2, the variety IT81D-994 is moderately resistant to the ecotype of *Alectra* from Koupela (Dieni et al., 2018). This genotype also showed differential reaction to *Alectra* in Nigeria (Singh et al., 1993; Omoigui et al., 2012). On the basis of these findings, the resistance genes in

genotypes KVx414-22-2 and IT81D-994 are considered to be different and non-allelic. Therefore, the symbol *Rav4* could be proposed for the resistance gene in KVx414-22-2. The variety KVx414-22-2 is an improved line from Burkina Faso possessing farmers' preferred traits (large and white seeds) but it is *Striga* and virus susceptible. Nevertheless, it can be an ideal donor parent for improving cowpea for both *Alectra* resistance and grain quality through backcross breeding.

5. Conclusion

The results of this study confirmed that the resistance to *Alectra vogelii* is conferred by two independent dominant genes in the genotype B301. On the other hand, the resistance is conferred by a single dominant gene in the variety KVx414-22-2. The three genes in the two varieties are non-allelic. The two genes in B301 have been named *Rav1* and *Rav2* and the gene present in IT18D-994 has been given the symbol *Rav3* as noted earlier. The symbol *Rav4* is therefore proposed to be the name of the resistance gene in genotype KVx414-22-2.

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