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SCREENING FOR RESISTANCE TO COWPEA APHID (*APHIS CRACCIVORA* KOCH) IN WILD AND CULTIVATED COWPEA (*VIGNA UNGUICULATA* L. WALP.) ACCESSIONS

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Abstract: Cowpea aphid (*Aphis craccivora* Koch) is a major pest of cowpea (*Vigna unguiculata* L. Walp.) particularly in the drier regions of the tropics. Although aphid resistant varieties had been developed, resistance has recently broken down. The present study seeks to identify new sources of resistance. 105 cowpea cultivars and 92 wild cowpea accessions were screened in a greenhouse for resistance to cowpea aphid. The results revealed that Among the cowpea cultivars only IT97K-556-6 showed some level of tolerance as 40 % of the seedlings survived until 21 days after infestation even with a high aphid population. The cultivar TVu 1659 had the highest number of aphids per seedling with 59.0 and 268.0 at 9 and 13 days after infestation, respectively while the wild cowpea accession TVNu 1158 had the lowest number of aphids per seedling with 3.2, 11.0 and 25.0 at 5, 9 and 13 days after infestation, respectively. Apart from having the lowest number of aphids 80% of the seedlings of this wild cowpea line survived to 21 days after infestation. The wild cowpea line TVNu 1158 was consistently aphid resistant and should be a good source of resistance genes for incorporation into cultivated cowpea.
Keys: Cowpea, *Aphis caccivora*, resistance, control.

INTRODUCTION

Cowpea (*Vigna unguicualata* L. Walp.) is an annual legume that is adapted to warm conditions and sensitive to low temperatures. It is an important food legume and an essential component of cropping systems in the drier regions of the tropics covering parts of Asia and Oceania, the Middle East, Southern Europe, Africa, Southern USA and Central and South America (Singh *et al.*, 2002). Nigeria, Brazil, Niger and Burkina Faso (FAO, 2008) are among the major producers and account for over 70 % of the world crop.

Cowpea is grown to produce dry grain, fresh southern peas, edible pods, edible leaves, hay or forage, or as a green manure crop (Anthony *et al.*, 1997). Cowpea grains contain between 17 and 32% protein on a dry weight basis and it is one of the cheapest sources of protein in the diets of peoples of West and Central Africa where cowpea is an important crop (Fatokun,

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2002). The consumption of even small amounts of cowpea ensures the nutritional balance of the diet. Cowpea production is constrained by many factors that are both biotic and abiotic. These factors are responsible for the generally low grain yield of cowpea across Sub-Saharan Africa in particular. Average grain yield has been reported to be about 324 kg/ha in the major cowpea growing countries of the world (Singh *et al.*, 2002). The low cowpea yields have been attributed to several factors which include the use of unimproved varieties, poor soil conditions, inadequate management practices, poor cultural practices and heavy biotic stresses, particularly from insects, diseases and parasitic weeds which often attack in the field and weevils that destroy seeds in storage (Rachie, 1985).

Every stage in the life cycle of cowpea has at least one major insect pest that can cause serious damage and impact yield negatively (Fatokun, 2002).The cowpea aphid, *Aphis craccivora*, is a major pest of cowpea in Africa (Singh and Jackai, 1985). It is considered as one of the important pests of cowpea in Africa, Asia and Latin America (Jackai and Daoust, 1986). In West Africa, during the last decade, aphid populations have continuously increased, consequently causing major losses (Singh *et al.*, 1990). Aphids primarily infest seedlings, although large populations also infest flower buds, flowers and pods. They cause direct damage to cowpea seedlings by sucking the sap and indirect damage by transmission of aphid-borne mosaic viruses. They have been implicated as the main vectors of the non-persistent cowpea aphid-borne mosaic virus (Atiri *et al.*, 1986). Small aphid populations have no major impact on cowpea production, but large populations can cause leaf distortion, stunting and poor nodulation of root systems (Singh and Jackai, 1985). Yield is reduced and, in extreme cases, the plant is killed (Singh and van Emden, 1979).

An indirect and generally the most harmful effect, even at low population densities, is the transmission and spread of legume viruses, which severely reduce yield (Singh and van Emden, 1979). At least 14 legume viruses are transmitted by *A. craccivora* (Thottappilly *et al.*, 1990).

Singh and Allen (1980) have reported a number of insecticides which are effective against the aphid, but insecticides are often not accessible to small-scale farmers who produce most of the cowpea. The use of resistant varieties appears to be the best option for the small-scale farmers of the semi-arid tropics owing to its low cost, compatibility with other control methods, and to the low income realised by farmers (Dent, 1991). Screening for aphid resistance has been conducted in IITA and several resistant lines have been identified (Singh and Jackai, 1985) and used in the breeding programmes to develop aphid resistant cultivars

(Singh and Ntare, 1985). However, the resistance to *A. craccivora* of all the identified cowpea cultivars at IITA has recently broken down (Fatokun personal correspondence). Therefore there is need to conduct new screenings in order to identify new sources of resistance to *A. craccivora.*

Thus, the objective of the present study was to identify cowpea cultivars and wild cross-compatible relatives with resistance to *A. craccivora.*

MATERIALS AND METHODS

The study was conducted in a greenhouse at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The materials consisted of 105 cultivated cowpea cultivars and 92 wild cowpea cultivars obtained from the cowpea breeding programme and the Genetic Resources Center (GRC) of IITA. Aphids used for this study were collected from Lafenwa and Papa in Ogun state, Osogbo, Ilesa and Ikirun in Osun state all located in southwest Nigeria. The aphids were maintained on a susceptible cowpea cultivar, TVx 3236 in a greenhouse away from parasites and/or other predators at IITA.

At the initial stage of the present screening, a relatively simple but reliable technique was used which consisted of scoring for dead (susceptible) and living (resistant) up to 21 days after infestation in order to significantly reduce the number of cowpea accessions being screened by early elimination of susceptible ones. Counting of aphid and scoring for the aphid population per plant were used in subsequent screening of accessions that appeared to be tolerant/resistant during the first 21-day screening. The experimental design used in the present study was Completely Randomized Design (CRD) with 5 replications.

Thus, for the first screening, seeds of 100 cowpea cultivars were sown on June 30, 2010 in wooden trays filled with sterilized top soil and kept in insect-proof cages with fine saran mesh, small enough to allow passage of air but not insects. Each tray contained five rows and each row had five plants of the same accession. Soil was irrigated with tap water as necessary. Seven days after planting, each seedling was infested with five aphids by placing them with a camel hair brush. The trays remained in the insect-proof cages for 21 days after which the plants were assessed for damage by aphids. Dead plants were regarded as susceptible while those still alive, with first trifoliate leaves developing, as resistant. On April 15, 2011 TVu 2672, TVu 8896, TVu 9797, TVu 436, TVu 12030 that appeared to have some level of tolerance during the initial screening along with five other lines not screened before (TVu 1659, IT99K-1060, IT99K-573-1, IT97K-556-6 and Vita 7) were planted in the

greenhouse and screened for aphid resistance. The same screening method as above was used, but in addition, aphids per plant were counted at 5, 9, 13, 17 and 21 days after infestation. Also the aphid population build- up on each plant was assessed using a 0 to 5 rating scale (0 = no aphids, 1= a few individual aphids, 2 = few small individual colonies, 3 = several small colonies, 4= large individual colonies, 5 = large continuous colonies). The score was taken at the same intervals as the count.

Accessions of wild cowpea (*Vigna unguiculata* subspecies *dekindtiana,* variety *congolensis, dekindtiana, grandiflora* and *pubescens*) used in this study were divided into two sets for screening. The first set consisting of 50 lines was sown on August 30, 2010 and the second set composed of 42 accessions was planted on September 23, 2010. The two sets were screened for aphid resistance using the same screening method as for the cowpea cultivars described above.

Plants that survived aphid infestations were transferred from the wooden trays to plastic pots and allowed to grow and produce seeds. Seeds harvested from individual plants were further tested for aphid resistance using the same screening method as above. Counting of aphids per plant was carried out and the aphid population build-up was scored with the same scale as for the cowpea cultivars.

Data analysis

Data collected were analyzed using descriptive statistics. Average number of aphids per accession was calculated and means level of infestation scores of each accession was determined. The count data collected was transformed using logarithm transformation. It was subjected to analysis of variance (ANOVA) using SAS 9.2 and treatment means separation was conducted using the Student-Newman-Keuls (SNK) method. Correlation (r) between average number of aphids per accession and the level of infestation was determined.

RESULTS

The rate of aphid population build-up on seedlings of the cultivated cowpea was very rapid and the plants were fully colonized within 7 to 10 days after infestation. The cowpea cultivars varied from one another in terms of number of aphids per plant. The cowpea line IT997K-573-1 recorded the highest number of aphids at 5 and 9 days after infestation with 89.0 and 268.4, respectively while TVu 1659 had the highest number (281.6) of aphids at 13 days. Lines IT99K-1060, TVu 8896 and Vita 7 had the lowest number of aphids at 5, 9 and13 days after infestation with 6.0, 51.5 and 87.0 per plant, respectively. IT97K-556-6 recorded the

highest number (240.0) of aphids at 17 days after infestation and it was the only surviving line at 21 days with 113.0 aphids.

The primary leaves turned yellow and started dropping from 10 days after infestation on susceptible plants. Among the cowpea cultivars only IT97K-556-6 showed some level of tolerance as 40 % (Table 1) of the seedlings survived at 21 days after infestation even though there was high aphid population on the seedlings.

Among the cultivars, there were significant differences in terms of number of aphids per seedling at 5 and 9 days after infestation (Table 2). However no significant differences were observed in the number of aphids among cultivated cowpea lines at 13 days after infestation. This is a reflection of the observed wide replicate to replicate variation within an individual accession as shown by the coefficient of variability i.e. 85.4%. At five days after infestation, IT99K-573-1, having the highest number of aphids i.e. 89.0 was significantly different from IT97K-556-6, IT99K-1060, TVu 2672 and TVu 8896 which had much fewer aphids i.e. 10.6, 6.0, 15.2 and 13.4, respectively (Table 2). However, there were no significant differences between IT99K-573-1 on one hand and TVu 436, TVu 1659, TVu 9797, TVu 12030, and Vita 7 on the other, five days after infestation. At nine days after infestation, TVu 8896 recorded the lowest number (51.5) of aphids but it was significantly different from only one of the remaining lines viz IT99K-573-1, which again had the highest number.

Table 1. Percentage survival of cultivated cowpea seedling from 5 to 21 days after infestation

	Days after infestation				
Accessions	5	9	13	17	21
IT97K-556-6	100	100	100	60	40
IT99K-573-1	100	100	60	0	0
IT99K-1060	100	100	100	0	0
TVu 436	100	100	100	0	0
TVu 1659	100	100	100	0	0
TVu 2672	100	100	100	60	0
TVu 8896	100	100	100	40	0
TVu 9797	100	100	50	0	0
TVu 12030	100	100	40	0	0
Vita 7	100	100	40	0	0

Table 2. Average number of aphids per cultivated cowpea seedling from 5 to 21 days after infestation.

	Days after infestation				
Accessions	5	9	13	17	21
IT97K-556-6	10.6c	67.9ab	200.7	240.0	113.0
IT99K-573-1	89.0a	268.4a	145.0	-[1]	-
IT99K 1060	6.0c	64.2ab	90.3	-	-
TVu 436	43.0abc	141.5ab	151.7	-	-
TVu 1659	59.0ab	227.6ab	281.6	-	-
TVu 2672	15.2bc	93.9ab	156.6	198.1	-
TVu 8896	13.4c	51.5b	174.6	198.1	-
TVu 9797	26.7abc	123.0ab	109.0	-	-
TVu 12030	33.6abc	126.0ab	81.7	-	-
Vita 7	21.9abc	104.5ab	87.0	-	-
			ns[2]	ns	

Means followed by a common letter are not significantly different at 0.01 level

[1] = no surviving plants

[2] = non-significant differences among surviving plants

In the case of wild cowpea, 10 lines (TVNu 1158, TVNu 303, TVNu 539, TVNu 432, TVNu 386, TVNu 912, TVNu 661, TVNu 294, TVNu 297 and TVNu 451) among the 92 accessions that were screened, survived during the first screening. However, TVNu 294, TVNu 297 and TVNu 451 were not included in the results of the present study because TVNu 294 and TVNu 451 had very poor germination while TVNu 297 did not produce seeds. The aphid population on the wild cowpea was low (Table 3) compared to that of cultivated lines (Table 2). Among the wild cowpea TVNu 912 had the highest number of aphids per plant from the beginning to the end of the screening with 15.0, 37.1, and 48.1 at 5, 9, and 13 days after infestation, respectively. It was followed by TVNu 432 with 14.4 aphids at five days after infestation. However, the second highest number was recorded by TVNu 303 with 35.5 and TVNu 432 with 40.7 aphids at 9 and 13 days after infestation, respectively. TVNu 1158 had the lowest number (Table 3) of aphids throughout the screening and it was also the only surviving line (Table 4) 21 days after infestation. Even though the number of aphids per

seedling varied from one line to another, no significant differences were detected among the wild accessions up to 13 days after infestation after which no accession survived to 17 days except TVNu 1158. The wild cowpea lines tested showed better levels of aphid tolerance when compared to the cultivated lines. This was reflected in the rate of aphid population build-up on their seedlings which was slower compared to those of cultivated lines (Fig. 1). A high rate of aphid population build-up was observed between 5 and 9 days after infestation for both cultivated and wild cowpea but the rate was higher in the cultivated lines. However, between 9 and 13 days after infestation, the rate of aphid population build-up was lower compared to that between 5 and 9 days. The decrease was greater in the case of the wild cowpea. The wild cowpea line TVNu 1158 consistently exhibited tolerance as aphid population reduced on the seedlings with time (Table 3) and 80% of its seedlings survived at 21 days after infestation (Table 4), it should be a good source of tolerance genes for incorporation into cowpea lines.

A positive correlation was observed between average number of aphids per plant and damage rating i.e. appearance of the plant, in the cowpea cultivars. A similar result was obtained for the wild cowpea.

Table 3. Average number of aphids per wild cowpea seedling from 5 to 21 days after infestation.

	Days after infestation				
Accessions	5	9	13	17	21
TVNu 303	7.5	35.5	26.0	-[1]	-
TVNu 386	4.1	29.3	39.0	-	-
TVNu 432	14.4	33.6	40.7	-	-
TVNu 539	10.5	23.6	28.7	-	-
TVNu 661	13.0	19.0	40.0	-	-
TVNu 912	15.0	37.1	48.1	-	-
TVNu 1158	3.2	11.0	25.0	13.2	6.3
	ns[2]	ns	ns		

[1] = no surviving plants

[2] = non-significant differences among surviving plants

Table 4. Percentage survival of wild cowpea seedling from 5 to 21 days after infestation

Accessions	Days after infestation				
	5	9	13	17	21
TVNu 303	100	50	50	0	0
TVNu 386	100	100	67	0	0
TVNu 432	100	100	100	0	0
TVNu 539	100	100	33	0	0
TVNu661	100	100	100	0	0
TVNu 912	100	100	100	0	0
TVNu 1158	100	100	100	100	80

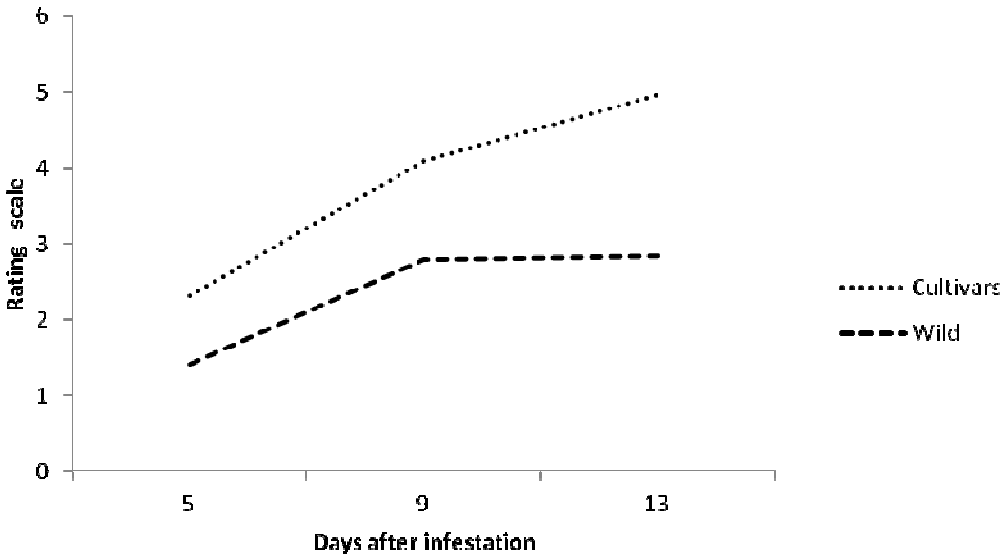

Fig. 1. Aphid population build-up on seedlings of cowpea cultivars and wild cowpea.

The correlation for the cowpea cultivars was highly significant (p = 0.01) between counts and rating at each of three occasions of assessment. In the case of the wild cowpea the correlation was significant (p= 0.01) only at the second and third occasions.

DISCUSSION

The multiplication of aphids on susceptible and tolerant plants was rapid and the plants were fully colonized within 7 to 10 days after infestation. There were significant differences in

terms of average aphid number per line at 5 and at 9 days after infestation for the cowpea cultivars. This finding is consistent with that of Arturo *et al.* (1988) who reported significant differences in the cumulative number of progeny per aphid female on the fourth day and onward in a cross between the susceptible line and the two resistant lines. Ofuya (1993) also reported significant differences in number of aphids on susceptible and resistance varieties. The leaves of susceptible accessions in the present study turned yellow became stunted and died between 10 to 15 days after infestation and this result agreed with that of Bata *et al.* (1987). The present study revealed that IT97K-556-6, a cultivated cowpea was moderately tolerant in terms of high number of aphids and relatively moderate survival rate while TVNu 1158, a wild cowpea was consistently aphid tolerant. This result is in agreement with the findings in India, of Jayappa and Lingappa (1988) who reported the cowpea cultivars Mandya local, P-912, P-1475, MS-370, TVu 2740 as tolerant and TVu 857, P-560, P-1473 and TVu 1948 as moderately tolerant to aphids. In the present study, tolerance is the mechanism of resistance found in IT97K-556-6 and TVNu 1158. However, this finding is contrary to that by Ofuya (1993) who reported seedling and pod resistance in TVu 9930, a cultivated cowpea as *A. craccivora* was completely absent from the pods of that variety two weeks after initial infestation of its young pods with the aphid. TVu 9930 was however, not screened in the present study. However, the present results indicating tolerance is also not consistent with that of Arturo *et al.* (1988) who reported only resistance which was expressed through antibiosis and antixenosis in cowpea lines ICV 11 and ICV 12 which were also not screened in the present study.

The present study revealed positive and significant correlation at each of the three occasions of assessment between the average aphid number per accession and damage i.e. level of infestation of the plant, in the cowpea cultivars. A similar result was obtained for the wild cowpea except that the correlation was significant only at the second and third occasions.

This is in agreement with the findings of Alabi *et al.*, 2003 who reported significant correlation between these two parameters in respect of cowpea thrips.

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REFERENCES

[1] Alabi, O. Y., Adebiyi, J. A. and Jackai, L. E. N. 2003. Field evaluation of cowpea cultivars [*Vigna unguiculata* (L) Walp.) for resistance to flower bud thrips (*Megalurothrips sjostedti* Trybom). *Inter. J. Pest Manag.,* 49 (4): 287-291.

[2] Anthony, E. H., Singh, B. B. and Jeffrey, D. E. 1997. Cowpea Breeding. *Plant Breeding Reviews, volume 15*. Jules, J. Eds. John Wiley and Sons, Inc.

[3] Arturo, G., Jens, W. and Jan, P. 1988. Cowpea aphid performance and behaviour on two resistant cowpea lines. *Entomol. Experi. Appl.,* 49: 259-264.

[4] Atiri, G. I., Enobakhare, D. A. and Thottapilly, G. 1986. The importance of colonizing and non-colonizing aphid vectors in the spread of cowpea aphid-borne mosaic virus in cowpea. *Crop prot.,* 5: 406-410.

[5] Bata, H. D., Singh, B. B., Singh, S. R. and Ladeinde, T. A. O. 1987. Inheritance of resistance to aphid in cowpea. *Crop Science* (27): 892-894.

[6] Dent, D. 1991. *Insect pest management*. CAB, Wallingford, UK. 604pp.

[7] F. A. O (Food and Agricultural Organization of the United Nations). 2008. FAOSTAT Data.

[8] Fatokun, C. A. 2002 Breeding cowpea for resistance to insect pests : attempted crosses between cowpea and *Vigna vexillata*. *Challenges and Opportunities for Enhancing Sustainable Cowpea Production*. *Proceedings of the World Cowpea Conference III.* 4-8th September 2000. C. A. Fatokun, S. A., Tarawali, B. B., Singh, P. M., Kormawa, and M. Tamo. Eds. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 52-61.

[9] Jackai, L. E. N and Daoust, R. A. 1986. Insect pests of cowpea. *Annual Review of Entomology* (31): 95-119.

[10] Jayappa, B. G. and Lingappa, S. 1988. Screening of cowpea germplasm for resistance to *Aphis craccivora* Koch in India. *Tropical Pest Management* (34) (1): 62-64.

[11] Ofuya, T. I. 1993. Evaluation of selected cowpea varities for resistance to *Aphis craccivora* Koch. (Homoptera: Aphididae) at seedling and podding phase. *Annals of Applied Biology* (123): 19-23.

[12] Rachie, K. O. 1985. Introduction. *Cowpea Research, Production and Utilization*: S. R. Singh and K. O. Rachie. Eds. John Wiley and Sons, New York. i-iv.

[13] Singh, B. B. and Ntare, B. R. 1985. Development of improved cowpea varieties in Africa. Cowpea Research, Production and Utilization. S. R. Singh and K. O. Rachie. Eds. John Wiley and Sons, New York. 105-116.

[14] Singh, B. B., Ehlers, J. D., Sharma, B. and Freire Filho, F. R., 2002. Recent progress in cowpea Breeding. Challenges and Opportunities for Sustainable Cowpea Production. Proceedings of the World Conference III. 4-8th September 2000. C. A. Fatokun, S. A., Tarawali, B. B., Singh, P. M., Kormawa, and M. Tamo. Eds. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 22-40.

[15] Singh, S. R and van Emden, H. F. 1979. Insect pests of grain legumes. *Annual Review of Entomology* (24): 255-278.

[16] Singh, S. R and Allen, D. J. 1980. Pests, diseases, resistance and protection of *Vigna unguiculata* (L.) Walp. *Advances in legumes science*. R. J. Summerfield and H. A. Bunting. Eds. London: Royal Botanical Gardens, and Ministry of Agriculture, Fisheries and Food. 419-443.

[17] Singh, S. R and Jackai, L. E. N. 1985. Insect pests of cowpea in Africa: their life cycle, economic importance and, potential for control. *Cowpea Research, Production and Utilization*. S. R. Singh and K. O. Rachie. Eds. John Wiley and Sons, New York. 217-231.

[18] Singh, S. R and Jackai, L. E. N. 1985. Insect pests of cowpea in Africa: their life cycle, economic importance and, potential for control. *Cowpea Research, Production and Utilization*. S. R. Singh and K. O. Rachie. Eds. John Wiley and Sons, New York. 217-231.

[19] Singh, S. R Jackai, L. E.N., Dos Santos, J. H. R. and Adalia, C. B. 1990. Insect pests of cowpea. *Insect pests of tropical food legumes*. S. R. Singh. Eds. Chichester: John Wiley and Sons Ltd. 43-89

[20] Thottapilly, G., Rossel, H. W., Reddy, D. V. R., Morales. F. J., Green, S. K. and Makkouh, K. M. 1990. Vectors of Virus and Mycoplasm diseases: an overview. *Insect Pests of Tropical Food Legumes*. S. R. Singh. Eds. Chichester: John Wiley and Sons Ltd. 323-342.