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Full Length Research Paper

Insect mediated outcrossing and geneflow in cowpea (*Vigna unguiculata* (L.) Walp): Implication for seed production and provision of containment structures for genetically transformed cowpea

Asiwe, J. A. N.

Agricultural Research Council (ARC), Grain Crops Institute, Private Bag X1251, Potchefstroom 2520, South Africa.
E-mail: AsiweJ@arc.agric.za. Fax: +27 18 294 7146.

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Insect mediated out-crossing poses a lot of problems in plant breeding and seed production by creating unwanted genotypes or off-types in the field including mixtures in seed lots. Removal of off-types in the field or seed lots increases costs of production. Information on level of out-crossing in cowpea and its insect pollinators as well as associated pollen movement would be useful in developing strategies to reduce outcrossing related problems. The objectives of this paper were to identify insect pollinators of cowpea, determine the level of outcrossing in the crop, and its implications in the deployment of genetically transformed cowpeas. Two experiments were conducted in Nigeria (IITA Ibadan and Mokwa) and Benin Republic (Cotonou) to assess geneflow frequency in cowpea under different planting arrangements. The first experiment was to quantify level of insect mediated out-crossing when three cowpea varieties (IT95K-1491, Sanzi, and IT86D-719) were respectively planted in alternate rows (one metre apart) with IT95K-1093-5. The second was to assess the level of insect mediated outcrossing when IT95K-1491, a breeding line with morphological marker (anthocyanin pigment), was planted in 3 - 4 concentric inner rows of 1 m apart while IT95K-1093-5 was planted in outer concentric rows up to 16 m from the epicenter. Incidence of insects visiting the plots was taken during the flowering stage of the crop. During harvesting, pods of IT95K-1093-5 were harvested from every plant within 3 - 4 m length along north, south, west and east axes of each concentric circle. In the case of alternate row planting, pods from single plants were harvested from rows of IT95K-1093-5 of each pair. Seeds derived from these plants (IT95K-1093-5) were planted in the field in order to assess the level of outcrossing that resulted from the movement of pollens from IT95K-1491, Sanzi, and IT86D-719 onto IT95K-1093-5 in the case of alternate row planting and IT95K-1491 onto IT95K-1093-5 for the concentric planting. Percentage of hybrids bearing the morphological trait of the marker parent was determined. Results obtained showed that level of out crossing was higher (0.5 to 0.85%) when cowpea was planted in alternate rows than in concentric rows (0.01 to 0.13%). Outcrossing was found to have occurred in a random fashion beyond 13 m. Among the insects observed, only honey and bumble bees were found with cowpea pollen dusts on their legs and abdomens, and were responsible for the observed level of outcrossing.

Key words: Cowpea varieties, geneflow, insect pollinators, out-crossing.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp, is the third important grain legume in South Africa after groundnut and dry beans. It is a major source of cheap plant protein to most poor families, provides regular income to farmers for the sale of grain and fodder, and a good source of animal fodder. In addition, cowpea is important for the sustainability of soil fertility. Cowpea exhibits different morphological forms; some are prostrate, erect or climbing. The leaves are trifoliate; inflorescences are axillary with few crowded flowers near the tip in alternate pairs. The anthers bear sticky and heavy pollen grains (Purseglove, 1984). The flowers open early in the morning and close before noon (Obong, 1977). The extrafloral nectaries at the base of the corolla attract insects (Mackie, 1946)

Cowpea is cleistogamous, producing viable pollens and receptive stigma before anthesis. This phenomenon imposes entirely self-pollination on the crop. However, outcrossing mediated by insects occur in nature. Different insect species visit cowpea flowers but not all are responsible for pollen movement associated with out-crossing. For genetic improvement purpose, hand or artificial pollination is necessary. The success of artificial pollination has been reported to be low ranging from 0.5 to 50% (Rachie et al., 1975) and varies with genetic and physiological factors as well as the care taken in handling floral parts during the process of emasculation (Umaharan et al., 1997; Obong, 1977). Under field situation, outcrossing by insects does occur. Literature available on outcrossing in cowpea is few. However, few past works were reported on other legumes. Kristofferson (1926) reports that 0.8 and 1.42% of outcrossing occurred in snap beans and field beans respectively. Insects, notably bumblebees and honeybees are reported to be responsible for outcrossing in *Phaseolus vulgaris* (Palmer, 1967). Contrary to Palmer's report, Mackie, and Smith (1935) assert that only thrips were responsible for the 0.73% outcrossing observed in common beans since thrips dusted with pollens from one flower can move into another flower of different variety before the flower opens.

Nevertheless, out-crossing in cowpea is often assumed by breeders to be very low and insignificant because cowpea is self-pollinated. Few studies have been conducted in this area probably because breeders and agronomists are discouraged to conduct such studies due to large area and plant population that are often needed for such investigation. Outcrossing poses a lot of problems to plant breeding and seed production by creating unwanted genotypes that constitute off-types in the field, and mixtures in seed lots. Rouging such off-types in field trials and/or removal of off-type seeds from seed lots increases costs of production. Knowledge of level of outcrossing in cowpea and the insects involved would help cowpea seed producers to evolve methods that would reduce its occurrence and associated problems, and will contribute to the advisory information for the deployment and management of genetically transformed (GT) cowpeas.

This paper reports on outcrossing, distance of pollen movement and associated insect pollinators in cowpea field.

MATERIALS AND METHODS

Two studies were carried out in the research farm of International Institute of Tropical Agriculture (IITA), Nigeria at two locations (IITA Ibadan and Mokwa) and Cotonou, Benin Republic, to assess geneflow frequency in cowpea. The first study was to quantify level of outcrossing when three cowpea varieties (IT95K-1491, Sanzi, and IT86D-719) were respectively planted in alternate rows (one metre apart) with IT95K-1093-5. The plot size was 20 rows by 10 m for each pair. IT95K-1491, Sanzi, and IT86D-719 have morphological markers, purple pigments at base of the petioles and ovate leaf shape while IT95K-1093-5 has no pigmented petioles and, the leaves are obvate and long. The second study was to assess the level of outcrossing when IT95K-1093-5 was planted several metres away from IT95K-1491. In this case, IT95K-1491 was planted in three concentric inner rows (hereafter referred to as epicenter for pollen movement) of 1m apart, and IT95K-1093-5 was planted in outer concentric rows up to 17 m from the epicenter. The plots were weeded as when necessary using hoes. Incidence of insects visiting the plots was observed during the flowering stage of the crop. Plots were sprayed regularly with insecticide to eliminate flower thrips, legume pod borer and pod suckers that could confound the expected data. During harvesting, pods of IT95K-1093-5 were harvested from every plant within 3 - 4 m length along north (ABFG), south (CDHI), west (ACLO) and east (BDJK) axes of each concentric circle (Figure 1). In the case of alternate row planting, pods from single plants were harvested from rows of IT95K-1093-5 of each pair. Seeds derived from these plants (IT95K-1093-5) were planted in the field in order to assess the level of outcrossing that resulted from the movement of pollens, that is, from IT95K-1491, Sanzi, and IT86D-719 onto IT95K-1093-5 in the case of alternate row planting and IT95K-1491 onto IT95K-1093-5 for the concentric planting. Percentage of hybrids bearing the morphological trait of the marker parent was determined.

The cowpea plot was fully protected with insecticide (Sherpa plus®). Incidence of bumblebees and other suspected insects were taken between 8:30 and 9:30 am during the flowering periods of the crop. Average time spent by pollen-mining insects on a flower was also noted. Flight pattern of the insects as they moved from one flower to another was also closely monitored. The insects were captured using sweeping net, and killed with household insecticide (Raid®). The captured insects were examined using binocular for the presence of cowpea pollens on their bodies.

RESULTS

Insect data

Insects captured during the flowering stage are shown in Table 1. The insects were grouped into two, pollinators, which consisted of insects found with cowpea pollens on their legs and abdomen and visitors which comprised insects without pollens on their body parts. The observed flight pattern of the insects was random. They could visit one, two or three flowers consecutively and flew far into the sky or nearby surrounding bush. Time spent by insects (pollinators) on cowpea flowers was observed to vary from 1 - 30 s.

Alternate row arrangement data

Out-crossing ranged between 0.50 and 0.85% (Table 2). Insect choice for visiting flowers was restricted to cowpea plants in alternate rows (dual-choice) which was one metre apart. Outcrossing was highest with IT95K-1093-5/IT95K-1491 (0.85%) followed by IT95K-1093-5 / SANZI (0.65%) while the least was obtained from IT95K-1093-5 / IT86D-719 (0.50%).

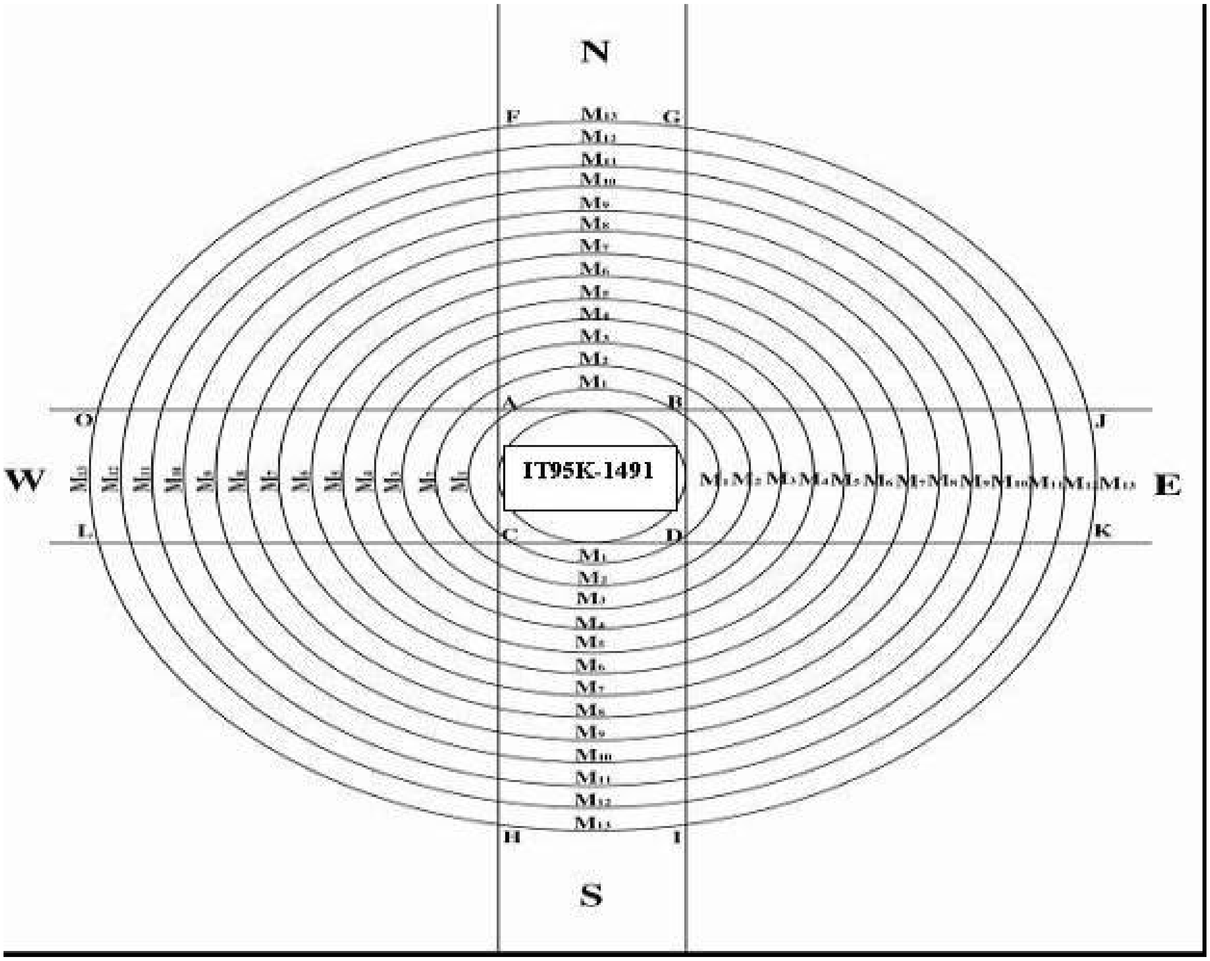

Figure 1. Concentric planting pattern used at Mokwa and Cotonou.

Table 1. Insect species captured in cowpea field during flowering.

Insects not associated with pollen movement (visitors)	Insects associated with pollen movement (pollinators)
Butterflies (Lepidoptera)	Carpenter bees (*Xylocopa virginica* L.)
Moths (Lepidoptera)	Digger bees (*Anthophora occidentalis* Cresson)
Mylabris spp.	Honey bees (*Apis mellifera* L.)
Oothecca mutabilis Sahlberg	Bumble bees (*Bombus grieocollis* De Geer)
Dragonflies (Odonata)	Bumble bees (*Bombus pennyslvanicus* De Geer)
Cotton stainer (*Dysdercus suturellus* Herrich Schäffer)	Leaf-cutting bees (*Megachile latimanus* Say)
Medythia quaterna Fairmaire	

Table 2. Cowpea varieties in alternate arrangements and their corresponding plant population and outcrossing at Ibadan, Nigeria.

Variety in alternate rows	Plant population	% Out-crossing
IT95K-1093-5/IT95K-1491	1893	0.85
IT95K-1093-5/IT86D-719	2577	0.50
IT95K-1093-5 / SANZI	1833	0.65

Table 3. Cowpea varieties in concentric arrangements and their corresponding plant population and percentage outcrossing in two locations, Mokwa (Nigeria) and Contonou (Republic of Benin).

Distance (m) from IT95K-1491	Mokwa		Cotonou	
	Plant population	% Out-crossing	Plant population	% Out-crossing
1	9110	0.09	14344	0.13
2	10186	0.00	16244	0.02
3	5593	0.02	10411	0.01
4	6010	0.03	8207	0.00
5	5001	0.00	6138	0.02
6	4221	0.00	7369	0.00
7	5855	0.05	8478	0.00
8	3410	0.09	11547	0.00
9	4006	0.00	11961	0.00
10	4618	0.00	13100	0.00
11	3858	0.00	8916	0.00
12	6187	0.02	11229	0.01
13	5042	0.00	17276	0.01
14	6516	0.09	.	.

$r^2 = -0.04$; $r^2 = -0.52$.

Concentric row arrangement

Outcrossing ranged between 0.02 and 0.09%, $r^2 = -0.04$ at Mokwa, Nigeria and 0.01 - 0.13%, $r^2 = -0.52$ at Cotonou, Republic of Benin (Table 3). In both locations, percentage outcrossing was lower than that obtained at Ibadan under the alternate row arrangement. The correlation between pollen movement by insects and distance from epicentre was very weak, $r^2 = -0.04$ at Mokwa, Nigeria and $r^2 = -0.52$ at Cotonou, Republic of Benin. Outcrossing was random and had no discernable relationship with distance away from the epicentre.

DISCUSSION

This study showed that only honey and bumble bees were responsible for the observed level of outcrossing in cowpea. This was because only heavy insects such as honey and bumble bees with powerful vibration from their wings could depress the wings of cowpea flowers and expose their stamens and stigmas for pollination. This confirmed the result obtained by Palmer (1967) in which he reports that bumblebees and honeybees were responsible for outcrossing in *P. vulgaris*. Contrary to Palmer's finding, Mackie and Smith (1935) reported that only thrips were responsible for the 0.73% outcrossing observed in common beans. In this study, the claim of Mackie and Smith could not be confirmed because flower thrips were eliminated with insecticide to avoid possible destruction of flowers pollinated through out-crossing. Role of flower thrips in pollen movement may need further studies under non-spray condition where other insect-pollinators would be excluded.

The study showed that percentage outcrossing in cowpea was low. However, in comparison between the two planting patterns, out-crossing was higher in the alternate than the concentric row planting, indicating that more mixtures or off-types would arise when different cowpea varieties are planted in proximate rows of one metre apart. This would increase cost of seed-sorting or removal of off-type in cowpea field. In more serious situation, it would increase the difficulty of identifying true genotypes or hybrids in a segregating population. Results obtained in this study are in conformity with past results obtained in other legumes. Report by Kristofferson (1926) shows that 0.8 and 1.42% outcrossing occurred in snap beans and field beans, respectively. In the case of the concentric pattern, the fact that outcrossing occurred in a random fashion beyond thirteen metres away from the epicentre also demonstrated the movement pattern of the pollen carriers (the insects). In other words, insect movement was random and hence, the out-crossing followed the same fashion, and had no direct bearing with distance away from the epicentre. For this reason, provision of containment structures for GT cowpea under experimentation and observation should not be based on isolated minimum distance, open containment structures at a given minimum distance from cowpea plots, or fencing. Rather, GT cowpeas being tested for a release should be given adequate protection from pollen carriers. Such GT cowpeas should only be planted in well-netted screen-houses to prevent the entrance or exit of bees or insects that are capable of moving pollens. In addition, to avoid or reduce outcrossing in cowpea experimental fields, habitats of bees such as spockets of bush around the cowpea fields should be destroyed or sprayed regularly with insecticides before cowpea flowers. As seen from the results of the alternate row arrangement, planting cowpea genotypes in such arrangement will enhance the chances of out-crossing. Seed multiplication of cowpea should aim at planting a variety in large plots in one location and not many varieties in alternate rows in a location.

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