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Full Length Research Paper

Reaction of transgenic Bt cowpea lines and their mixtures under field conditions

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Transgenic cowpeas are those genetically engineered with *Cry1Ab* gene for protection against the lepidopteran pest *Maruca vitrata* that could inflict upto 80% yield loss in severe infestation. Two transgenic events TCL-709 and TCL-711 were crossed to three non -transgenic lines IT97K-499-35, IT93K-693-2 and IT86D-1010 to generate six set of F_1 hybrids. The study was designed and conducted to evaluate the performance of transgenic cowpeas and hybrids derived from them under natural field conditions. The transgenics, non-transgenics and the hybrids were planted with intra and inter-row spacing of 30 and 75 cm respectively in a completely Randomized Block Design during the 2012 cowpea growing season at Confined Field Trial site of Institute for Agricultural Research of Ahmadu Bello University Samaru-Zaria. The plot sizes consisted of 2 rows of 5 m long. The trial was kept weed-free by constant manual weeding. The various generations of IT97K-499-35 x TCL-709 were infested six times with first instar *Maruca* larvae at four day interval at flower initiation stage while the remaining crosses were exposed to natural infestation of *Maruca*. The data were recoreded on various characters and the results revealed that the cowpea lines containing the *cry1Ab* gene produced enough toxin to kill and inhibit the feeding of *Maruca* on cowpea plants. The result on infestation of *Maruca* larvae scored as number of damaged pods per plant gave a clear proof of the achievement of the goal of the genetic transformation of cowpea lines with *Cry1Ab* gene. As the *Cry1Ab* gene conferred a high degree of resistance to cowpea pod borer, the transgenic cowpea lines should be used as a precious insect-resistant line to be employed in traditional breeding programs to develop *Maruca* resistant cowpea varieties.

Key words: *Bacillus thuriengiensis,* confined field trial, *Cry1Ab gene,* transgenic cowpea, *Maruca vitrata.*

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is an annual legume which originated in Africa and widely grown in Africa, Latin America, Southeast Asia and in the southern United States (Davis et al., 1991). It is chiefly used as a grain crop, for animal fodder and as a vegetable. It is considered the most important food grain legume in the dry savannas of tropical Africa (NGICA, 2002) and the most important indigenous African legume for both home use and as a cash crop (Kushwaha et al., 2004). Nearly 200 million people in Africa consume the crop (AATF,

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Table 1. Six combinations of crosses.

Cross	Female parent	Male parent
1. IT97K-499-35 x TCL-709	IT97K-499-35	TCL-709
2. IT97K-499-35 x TCL-711	IT97K-499-35	TCL-711
3. IT93K-693-2 x TCL-709	IT93K-693-2	TCL-709
4. IT93K-693-2 x TCL-711	IT93K-693-2	TCL-711
5. IT86D-1010 x TCL-709	IT86D-1010	TCL-709
6. IT86D-1010 x TCL-711	IT86D-1010	TCL-711

2010; NGICA, 2002). It is consumed in many forms; the young leaves, green pods, and green seeds are used as vegetables while dry seeds are used in various food preparations, the haulms are fed to livestock as nutritious supplement to cereal fodder and being a fast growing crop, cowpea curbs erosion by covering the ground, fixes atmospheric nitrogen, and its decaying residues contributes to soil fertility (Singh et al., 2002).

An estimated 14.5 million ha of land is planted to cowpea each year worldwide; with total annual production being approximately 6.2 million metric tonnes (MT) (Abate et al., 2012). Nigeria is the largest producer and consumer of cowpea with about 5 million (ha) and over 2 million tonnes produced annually (Singh et al., 2002). The overall productivity of its existing traditional genotypes are low due to their prominent susceptibility to insect pests and pathogens (Darshna et al., 2007). The cowpea pod borer *(Maruca vitrata)* is a serious lepidopteran pest that inflicts severe damage to cowpea on farmers' fields. In severe infestations yield losses of between 70 to 80% have been reported (AATF, 2010). Control through spraying with insecticide has not been fully adopted by farmers due to the prohibitive costs, causing resource-poor farmers to opt for cheaper but more toxic alternatives that impact their health (AATF, 2010). In addition, most farmers are not well equipped to protect themselves when using such toxic chemicals, in some farming communities, adulterated chemicals that do not control the pests are sold to farmers (Fatokun, 2009). Although great progress has been made in cowpea improvement, there is a need to protect the cowpea crop with 2–3 sprays of insecticide from *Maruca* pod borer for which no resistance has been found (AATF, 2010), so there is need to acquire a resistant variety.

Laboratory studies have shown that the *Cry1Ab* transgene derived from the *Bacillus thuriengiensis Bt* gene confers resistance on cowpea to the pod borer (Fatokun, 2009; Higgins, 2007) and the transfer of the *Cry1Ab* transgene into improved cowpea varieties, will reduce the need for insecticide sprays in cowpea and smallholder farmers can substantially increase their yields and greatly enhance their nutritional and economic status (AATF, 2010). This led to genetic transformation of an advanced breeding line (IT86D-1010) with *Cry1Ab* gene at the Commonwealth Scientific and Industrial Research Organization Laboratory, Canberra, Australia (Fatokun, 2009; Ishiyaku, 2010). Therefore, this study was conducted to evaluate the response of some genetically engineered cowpea lines with *Cry1Ab* transgene and their progenies (F_1) derived through hand-crossing under natural field conditions.

MATERIALS AND METHODS

The research was conducted under the Confined Field Trial site (CFT) of Institute for Agricultural Research (IAR), Ahmadu Bello University Zaria (ABU) between July, 2011 to August, 2012. Two genetically engineered cowpea line; cowpea transgenic line TCL-709 and TCL-711 and three non-transformed cowpea genotypes; IT97K-499-35, IT93K-693-2 and IT86D-1010 were used in this study. The descriptions of parental materials used are given in (Table 2).

Development of the genetic population

The cowpea transgenic lines TCL-709 and TCL-711 along with three non-transgenic genotypes; IT97K-499-35, IT93K-693-2 and IT86D-1010 (the original parent of the transformed lines having the same genetic architecture except the *cry1Ab* gene) were crossed using biparental mating as described by Sharma (2006) to generate six set of F_1 populations. The hybridizations were done as described by (Ehlers and Hall, 1997; Myers, 2006; Timko et al., 2007) and reported by Mohammed et al. (2012) by emasculating late in the evening and pollinating in the next morning. The following six combinations of crosses were made in Table 1.

Field evaluation

The parents and F_1 progenies were evaluated under confined field conditions during the 2012 raining season at CFT Samaru. The trial was planted using Randomized Complete Block Design with three replications. The plant to plant and row to row spacing was kept at 30 by 75 cm respectively. The plot size was 3 × 5 m for all entries. Field management was conventional except that no lepidopteran insecticide was applied throughout the experiments. NPK fertilizer 15:15:15 was applied with the amounts of 100 kg/ha two weeks after plant and the field was kept weed free.

Field bioassay with *Maruca* larvae

Artificial infestation with 1st instar larvae of *M. vitrata* was used to substantiate the *Bt* strips test for the classification of the genotypes as either resistant or susceptible (Mohammed et al., 2013). The generations (parents and F_1 plants) of a cross of IT97K-499-35 x TCL-709 were subjected to artificial infestation of *Maruca* larvae while other crosses were subjected to natural infestation of *Maruca*. For the purpose of field infestation, a large number of *Maruca* larvae were reared in the *Maruca* rearing laboratory situated in the Department of Crop Protection, ABU Zaria. Six infestation events were carried out at the interval of four days each. Ten 1st instar larvae, pre-fasted, were placed on a flower of each of the F_1 and parentals and were allowed to feed on the plants. Different levels of pressure were applied to the plants with 1 st instar *Maruca* larvae viz; 10 larvae/plant for the first infestation, 20 larvae/plant second infestation and 30 larvae/plant for third to the sixth infestation events. Larvae were placed on flowers of the plants with the help of soft hair brush; larvae were allowed to feed and

Table 2. The pedigree and description of the parental materials used.

Genotypes	Pedigree	Description
TCL-709	Transformation event derived from IT86D-1010.	Sourced from Commonwealth Scientific and Indutrial Research Organization laboratory, Canberra - Australia, transgenic line resistant to M. Vitrata (Higgins, 2007).
TCL-711	Transformation event derived from IT86D-1010.	Sourced from Commonwealth Scientific and Indutrial Research Organization laboratory, Canberra - Australia, transgenic line resistant to M. Vitrata (Higgins, 2007).
IT97K-499-35	Derived from a cross of IT93K-596-9-12 x IT93K-2046-1	It is a medium maturing variety (about 75 days) with semi-erect growth habit. Its heat tolerant and photo-insensitive variety with erect growth habit. It has large white seeds (about 18g 100 seeds-^{1}). In addition to being resistant to Striga and Alectra, it has combined resistance to major diseases and insect pests (Singh et al., 2006).
IT86D-1010	Derived from a cross between TVx4659-03E x IT82E-60	It is an advanced breeding line, medium maturity (71 days), photo-insensitive variety with semi- erect plant growth habit. It has combined resistance to cowpea yellow mosaic, blackeye cowpea mosaic and many strains of cowpea aphid borne mosaic, Cercospora, smut, rust, Septoria, scab, Ascochyta blight, bacterial blight, anthracnose, nematodes, Striga, Alectra, ahpid, thrips and bruchid (Lale and Kolo, 2007).
IT93K -693-2	IT88D-867-11 x IT89KD-374-57	IT93K-693-2 is resistant to Alectra as well as all five strains of Striga reported in West Africa (Singh, 2002). IT93K-693-2 is an extra-early maturing (about 60 days) photo-insensitive and heat tolerant variety with semi-erect growth habit. It has green plants without purple pigmentation. It has medium size seeds (about 14g 100 seeds- ^{1}) with brown color and rough seed coat texture. It has combined resistance to major diseases and insects (Singh et al., 2006).

developed to pupae in the field, grow into adult, lay eggs and damage the plants at larval stage. The infestation started on the 45th day after planting and continued till the sixth infestation event on the 69th day after planting. The data on insect damaged were taken on the 77th day after planting. The plants were categorized as:-Resistant plants (0% pod damaged) or susceptible plants (1 to 100%) pod damaged. A pod which was completely or partially damaged by any *Maruca* larvae was counted damaged while pods which were totally undamaged were counted as healthy/undamaged pods.

Data collection

Data were taken for further analysis on the following; Days to first flowering, Days to first pod maturity, Plant height at maturity, Number of primary branches at the vegetative stage, Total number of pods per plant, Number of damaged pods by *Maruca* larvae per plant.

Statistical analysis

Analysis of variance for parental genotypes and F_1 hybrids evaluated at the CFT 2012

The data pertaining to number of pods damaged due to infestation of *Maruca* larvae, total number of pods per plant, days to first flowering, days to first pod maturity and number of primary branches at vegetative stage per plant were subjected to analysis of variance using the generalized linear model (GLM) procedure of the statistical analysis system programme (SAS 9.0). The Least Significant Difference Test (LSD) was used to separate the means where there was significant difference. The standard linear model for an RCBD with both the block and treatment effects fixed as follows was used: $Y_{ij} = \mu + R_i + B_j + E_{ij}$, where Y_{ij} = denotes the response for the experimental unit with the i^{th} treatment in the j^{th} block, μ = is the overall mean, R_i = is the treatment effect, B_j = is the block effect, and E_{ij} = is the random error with i =1,…,a and j = 1,…,b. a = the number of treatments; b = the number of blocks (Shieh and Jan, 2004).

RESULTS

Evaluation of the transgenic lines and their hybrids at CFT Samaru in 2012

The result revealed that the genotypes had highly significant differences among themselves (Table 3). The results of mean performance of the parental materials and their hybrids used in this study are presented in

Table 3. Mean squares for different characters of the field evaluation at CFT 2012.

Source of variation	Days to first flowering	Days to first pod maturity	Plant height (cm)	Total number of pods	Number of primary branches	Number of pods damaged
Replication	1.27^{NS}	42.95**	7805.65*	1326.52*	0.66^{NS}	14.12^{NS}
Genotypes	28.27**	78.89**	31445.15**	6193.75**	2.09*	322.61**
Error	3.98	5.38	1624.36	391.50	0.91	7.79

*****Indicates significant differences at P<0.05 probability level, ** Indicates significant differences at P<0.01 probability level, NS indicates non significant difference.

Table 4. Mean performance of the parental materials and F_1 hybrids evaluated under natural infestation of *Maruca* at CFT, Samaru, 2012.

Genotypes Used	Days to first flowering	Days to first pod maturity	Plant height (cm)	Total number of pods per plant	Number of primary branches per plant	Number of pods damaged per plant
IT86D-1010	44.73	64.28	160.28	34.29	5.13	11.07
TCL-709	45.96	64.48	137.29	43.79	5.04	0.00
TCL-711	47.65	69.25	82.99	26.00	5.40	1.92
IT97K-499-35	48.40	71.10	56.94	23.00	5.20	5.36
IT93K-693-2	48.93	68.70	24.79	19.91	4.13	5.00
IT86D-1010 x TCL-709	46.62	65.27	125.81	35.00	5.07	0.08
IT86D-1010 x TCL-711	46.93	66.47	106.51	39.08	4.60	0.00
IT97K-499-35 x TCL-709	45.87	67.80	120.75	69.03	5.33	0.04
IT97K-499-35 x TCL-711	46.28	69.00	133.75	45.95	5.28	0.00
IT93K-693-2 x TCL-709	47.60	68.45	56.40	58.64	5.00	0.00
IT93K-693-2 x TCL-711	44.87	63.43	52.75	56.10	4.93	0.08
LSD	1.40	1.83	28.67	12.21	0.67	1.78

(Table 4). The mean number of days to first flowering (DFF) showed significant differences among the parental lines as well as the hybrids, the DFF ranged approximately from 45 to 49 days for IT86D-1010 and IT93K-693-2 respectively (Table 4). The DFF was early in IT86D-1010, which was not statistically different from TCL- 709, IT97K-499-35 x TCL-709, IT97K-499-35 x TCL-711, IT93K-693-2 x TCL-711. The F1 generations of IT86D-1010 x TCL- 709 and IT86D-1010 x TCL- 711 had medium number of days to first flowering. The days to first flowering was later in IT93K-693-2 and was not significantly different from IT97K-499-35, TCL-711, and IT93K-693-2 x TCL-709 (Table 4) (Samaru, 2012).

The days to first pod maturity (DFPM) showed significant differences among the genotypes (Table 4). It ranged from 63 to 71 days after planting for IT93K-693-2 x TCL-711 and IT97K-499-35 respectively. The DFPM was lowest in IT93K -693-2 x TCL-711, which was statistically not different from DFPM of IT86D-1010, TCL-709, IT86D-1010 x TCL-709. Genotypes TCL-711, IT93K-693-2, IT97K-499-35 x TCL-711, IT93K-693-2 x TCL-709, IT97K-499-35 x TCL-709, were statistically the same in regards to DFPM while IT97K-499-35 had the highest number of DFPM among the genotypes comaperd (Table 4). Similarly, the plant height showed significant differences among the genotypes comapared. Plant height ranged from 24.79 to 160.28 cm for IT93K- 693-2 and IT86D-1010 respectively. The plant height of IT86D-1010 was not statistically different from TCL-709, IT86D-1010 x TCL-709, IT86D-1010 x TCL-711, IT97K-499-35 x TCL-709 and IT97K-499-35 x TCL-711 while the plant height of IT93K-693-2 was not significantly different from that of IT93K-693-2 x TCL-711 (Table 4).

Statistical significant differences existed between the total number of pods per plant, it ranged from 23 to 69 for IT97K-499-35 and IT97K-499-35 x TCL-709 respectively. IT86D-1010 was not signifcantly different from TCL-709, TCL-711, IT97K-499-35, IT93K-693-2, IT86D-1010 x TCL-709, IT86D-1010 x TCL-711 and IT97K-499-35 x TCL- 711 in regards to mean total number of pods per plant while IT97K-499-35 differed statistically from IT93K-693-2 x TCL-709 (Table 4). The number of primary branches per plant taken at vegetative stage was statistically the same among all the genotypes compared (Table 4) while the number of pods damaged per plant due to infestation of *Maruca* was statistically the same for the two transgenic lines and all the F_1 hybrids while the IT97K-499 -35, and IT93K- 693-2 were the same in regards to pod damaged but differed in the degree of pod damaged from IT86D-1010 (Table 4). The non-transgenic

genotypes sustained various degree of pod damaged due to infestation of *Maruca* larvae (Table 4).

DISCUSSION

Field evaluation of the transgenic lines and hybrids for some key parameters

In the present studies, the non transgenic cowpea lines had higher number of pods damaged by *Maruca* larvae, these were similar to the results already reported by various workers, a few of which are described thus; Perlak et al. (1990) and Barton (1989) reported total protection from lepidopteran insect damage of leaf tissue of cotton plants expressing *Cry1Ab* and *Cry1Ac* genes. Ishiyaku et al. (2010) reported complete protection of some transgenic cowpea lines expressing *Cry1Ab* gene from *Maruca* damage under field conditions. Sachs et al. (1998) reported that cotton plants of different genetic backgrounds that possessed the *Cry1Ab* insecticidal protein were more resistant to tobacco budworm larvae than plants with other traits. In addition, Estruch et al. (1997), Halcomb et al. (1996), and Van-Rie (2000) evaluated plant stands of *Bt* cotton and non-*Bt* cotton, they found that the attack of bollworms and tobacco budworm was less on *Bt* cotton plants as compared to non-*Bt* cotton plants.

Conclusion

The performance of the transgenic cowpea lines and their hybrids in comparison with the non-transgenic parents regarding resistance to *Maruca* were conducted. The mean performance clearly indicated that the transgenic cowpea lines and hybrids to be better in insect resistance than the non- transgenic cowpea lines. The transgenics and the hybrids derived from them through hand crosses with the non-transgenic parents were statistically the same regarding the number of pod damaged due to infestation of *Maruca* larvae. It therefore, means that the two transgenic lines used and F_1 generations derived from them were resistant to *Maruca* while the non-transgenic genotypes sustained various degrees of damaged as a result of feeding activities by the *Maruca*. It is therefore, concluded that transgenic cowpea lines can be use as precious source of resistance to *Maruca* pod borer in the development of *Maruca* resistant cowpea varieties for the benefit of African farmers.

Conflicts of Interests

The authors have not declared any conflict of interests.

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