Full Length Research Paper

Application of Cry1Ab/Ac Bt strip for screening of resistance for Maruca vitrata in cowpea

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Maruca vitrata is a significant constraint to cowpea production in most cowpea growing areas of sub-Saharan Africa. Yield losses caused by M. vitrata in these regions are estimated in millions of tons annually and the prevalence of M. vitrata infestation is steadily increasing. Recombinant DNA technology have led to development of some cowpea lines with Maruca resistance as well as other important agronomic traits but it is time-consuming and difficult to screen for the resistant trait especially in the segregating populations using conventional screening techniques, which will lead to delay in the development of Maruca resistant cowpea varieties. The use of allele-based selection tool will make it easier to select plant traits and reduce the time needed to develop new Maruca resistant cowpea varieties. In this study, the efficacy of using Cry1Ab/Ac Bt strip for detecting Maruca resistant transgene in transgenic cowpea was systematically investigated for the first time through field derived progenies. The results show that the Cry1Ab/Ac Bt strip was effective for detecting the presence of the resistant gene in cowpea genome. Maruca resistant plants were successfully screened from the segregating cowpea plants and the genetics of the gene was monitored. The Cry1Ab/Ac Bt strip was found to be suitable for genetic analysis of the Maruca resistant transgene in cowpea. This study has demonstrated the precision of using Cry1Ab/Ac Bt strips as a screening tool of transgenic lines containing Cry1Ab gene, this has an importance in the hybridization programme where genotypes having cry gene can be distinguished at seedling stage at lesser time, with the potential of putting the breeding process on a fast track and increase the efficiency of breeding activities.

Key words: Bacillus thuringiensis, Cry1Ab/Ac Bt strips, transgenic cowpea, Maruca vitrata.

INTRODUCTION

Cowpea (Vigna unguiculata L. Walp) is considered the most important food grain legume in the dry savannas of tropical Africa (NGICA, 2002). It is the most important indigenous African legume for both home use and as a cash crop and especially important for the Sahel because of its drought tolerance (Kushwaha et al., 2004). It is rich in quality protein and has energy content almost equivalent to that of cereal grains, it is a good source of quality fodder for livestock and also provides cash income (Davis et al., 1991). Nearly 200 million people in Africa consume the crop (AATF, 2010; NGICA, 2002). Cowpea is consumed in many forms; the young leaves, green pods, and green seeds are used as vegetables, 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 contribute to soil fertility (Singh et al., 2002).

The overall productivity of its existing traditional geno-
types are low due to their prominent susceptibility to insect pests (Darshana et al., 2007) and among the most damaging insects are aphids, flower thrips, cowpea pod borer, pod sucking bugs and the cowpea weevils (Darshana et al., 2007). The cowpea pod borer (Maruca vitrata) is a serious lepidopteran pest that inflicts severe damage to cowpea on farmers’ fields (Figure 3). In severe infestations, yield losses of between 70-80% 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).

Breeding for insect resistance with the aid of phenotypic selection is time consuming, laborious and relatively expensive (Xu and Crouch, 2008). In addition, most crops have a high level of heterozygosity that makes visual selection difficult but selection based on allele composition will avoid this problem (Ibitoye and Akin-Idowu, 2010). Ability to select breeding progeny early at the seedling stage is another advantage of using allele-based selection tools (Ibitoye and Akin-Idowu, 2010). The number of plants that are needed to be maintained in a crop breeding programme can be reduced by eliminating progenies that do not carry the desirable allele at the seedling stage, saving space, time, labor and other resources (Ibitoye and Akin-Idowu, 2010). The present study was designed and conducted in order to understand the efficacy of using Cry1Ab/Ac Bt strips for detecting Maruca resistant transgene in transgenic cowpea through field derived progenies.

MATERIALS AND METHODS

The Research was conducted under the confined field trial site (CFT) between July, 2011 to August, 2012 at the Institute for Agricultural Research (IAR), Samaru-Zaria, Nigeria. Two genetically engineered cowpea lines: Transgenic cowpea 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. Data were collected as scores of Cry1Ab Bt strip test.

Cry1Ab inheritance studies

To establish the potency of Cry1Ab/Ac Bt strips as a screening tool for Maruca resistant transgene, the inheritance of Cry1Ab gene was monitored with the aid of Bt strips in filial generations.

Development of the genetic population

The transgenic cowpea 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 F1 population. Some F1 seeds were advanced to second filial generation (F2) populations by self pollination. The following six combinations of crosses were produced: IT97K-499-35 x TCL-709, IT97K-499-35 x TCL-711, IT86D-1010 x TCL-709, IT86D-1010 x TCL-711, IT86D-1010 x TCL-709 and IT86D-1010 x TCL-711.

Field evaluation

The parents, F1 and F2 generations were evaluated under field conditions during the 2012 cowpea growing season at CFT Samaru-Zaria between June to August, 2012. 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 x 5 m for all entries except F2 plants which were 6 x 5 m. No insecticidal spray against lepidopteran insects was applied.

Test procedure

The screening was carried out with the aid of Cry1Ab/Ac Bt strips to check for the presence of Cry1Ab gene in the genetic populations (P1, P2, F1 and F2) of transgenic cowpea. Detection of Cry1Ab proteins on cowpea involved assaying plant leaves for expression of the Cry1Ab gene. A quick Bt strip test was used to confirm the expression of the Cry1Ab protein in cowpea transgenic lines. This was achieved by placing leaf discs in test tubes containing buffer and then slowly inserting Bt strips into the buffer. Then, formation of a single line in the test tube proved that the test was working while the appearance of a second lower line showed that Cry1Ab protein was present (Envirologix, 2008). Figures 1 and 2 illustrate a typical type of Cry1Ab Bt strip test. In these figures, the appearance of two lines on the test membrane indicates the presence of the Cry1Ab Bt gene, while the appearance of only the top (control) line indicates a negative response.

Cry1Ab gene screening in F1 generation

The plants were screened with the aid of Cry1Ab/Ac Bt strips and the transfer of Cry1Ab gene from a transgenic cowpea plant to a non-transgenic cowpea plant was checked. The number of positive and negative plants indicating presence and absence of the transgene respectively, were taken to infer the behaviour of the transgene whether dominant or recessive and establish the efficacy of the Cry1Ab/Ac Bt strips.

Cry1Ab gene screening in Segregating Generations

Adequate sample size was taken from each F2 family and analyzed with the aid of Cry1Ab/Ac Bt strips. Since the gene is expected to segregate in F2 generations, the plants were clearly classified as Cry1Ab-positive or Cry1Ab-negative regarding the Cry1Ab expression where Cry1Ab positive plants indicates resistance to M. vitrata while Cry1Ab negative plants indicates susceptibility to M. vitrata. Envirologix (2008) procedures for Cry1Ab/Ac Bt strip test was carefully followed. The data was subjected to Chi-square goodness of fit test against the Mendelian ratio 3:1 for the F2 generations (Kiani et al., 2009).

Statistical analysis

Data recorded for the genetic segregation of Cry1Ab transgene were analyzed with the help of Chi-square (X2) goodness of fit test, to determine whether the observed data conforms to the expected Mendelian 3:1 ratios for F2 segregating populations of each cross. The following formula was used using a Proc Frequency for a chi-square test of goodness of fit by Mcdonald (2009):

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$
**Figure 1.** Cry1Ab/1Ac Bt strips showing positive, negative and invalid result (Envirologix, 2008).

**Figure 2.** Cry1Ab/Ac Bt strips in test tubes showing positive results.

**Figure 3.** Showing larvae and adult *Maruca vitrata* (legume pod borer) pest.
Screening for Cry1Ab transgene in F1 generations

The results of the six set of F1 plants analyzed with the aid of Cry1Ab Bt strips to study the efficacy of Bt strips for detecting the transgene’s presence through transmission and expression of the transgene are given in Table 1. It was found that all the F1 plants were positive to Cry1Ab Bt strip test. It thus means that the gene was successfully transferred from Bt lines to non-Bt lines and the Cry1Ab Bt strips were potent as detecting tool for the target gene.

Screening for Cry1Ab transgene in segregating populations

As shown in Table 2, it reveals that the Mendelian segregation ratios (3:1) existed in all the six cross combinations for the F2. The F2 populations of these crosses segregated into plants with positive and negative Cry1Ab gene indicating the presence and absence of the Maruca resistant gene, respectively, with a good fit to the Mendelian ratio of 3:1 with non significant Chi-square values (X^2) for F2 plants of the following crosses; IT97K-499-35 x TCL-709 (X^2 = 0.18 ; P = 0.67), IT97K-499-35 x TCL-711 (X^2 = 0.15 , P = 0.70), IT93K-693-2 x TCL-709 (X^2 = 0.22 ; P = 0.64), IT93K-693-2 x TCL-711 (X^2 = 0.31 ; P = 0.58), IT86D-1010 x TCL-709 (X^2 = 0.26 ; P = 0.61), IT86D-1010 x TCL-711 (X^2 = 0.0041 ; P = 0.95) (Table 2). This has demonstrated the potency of the Bt strips for detecting the presence of the transgene in the segregating populations of transgenic cowpea crosses. The strip screening clearly grouped the F1 plants as resistant plants just like the transgenic parents and the segregating progenies of F2 were seen clearly behaving as hypothesized into 3:1 Mendelian test ratio.

RESULTS

Detection of Cry1Ab gene in Parents and F1 populations of transgenic cowpea.

| Genotype       | Number of plants tested | Positive | Negative | Expected ratio |
|----------------|-------------------------|----------|----------|----------------|
| TCL-709        | 50                      | 50       | 0        | 1:0            |
| TCL-711        | 50                      | 50       | 0        | 1:0            |
| IT97K-499-35   | 25                      | 0        | 25       | 0:1            |
| IT93K-693-2    | 25                      | 0        | 25       | 0:1            |
| IT86D-1010     | 25                      | 0        | 25       | 0:1            |
| IT86D-1010 x TCL-709 | 23       | 23    | 0        | 1:0            |
| IT86D-1010 x TCL-711 | 23       | 23    | 0        | 1:0            |
| IT97K-499-35 x TCL-709 | 30    | 30    | 0        | 1:0            |
| IT97K-499-35 x TCL-711 | 28   | 28    | 0        | 1:0            |
| IT93K-693-2 x TCL-709 | 25   | 25    | 0        | 1:0            |
| IT93K-693-2 x TCL-711 | 23  | 23    | 0        | 1:0            |

Positive, Cry1Ab gene is present, that is, resistant to M. vitrata; negative, Cry1Ab is absent, that is, susceptible to M. vitrata.

| Cross (female x male) | No. of plants tested | Positive | Negative | Expected ratio | Chi-square | DF | Probability |
|-----------------------|----------------------|----------|----------|----------------|------------|----|-------------|
| IT86D-1010 x TCL-709  | 105                  | 81       | 24       | 3:1            | 0.26       | 1  | 0.61**      |
| IT86D-1010 x TCL-711  | 81                   | 61       | 20       | 3:1            | 0.004      | 1  | 0.95**      |
| IT97K-499-35 x TCL-709| 89                   | 65       | 24       | 3:1            | 0.18       | 1  | 0.67**      |
| IT97K-499-35 x TCL-711| 111                  | 85       | 26       | 3:1            | 0.15       | 1  | 0.70**      |
| IT93K-693-2 x TCL-709 | 75                   | 58       | 17       | 3:1            | 0.22       | 1  | 0.64**      |
| IT93K-693-2 x TCL-711 | 131                  | 101      | 30       | 3:1            | 0.31       | 1  | 0.58**      |

Positive, Cry1Ab gene is present, that is, resistant to M. vitrata; negative, Cry1Ab is absent, that is, susceptible to M. vitrata; ns, not significant at p=0.05.

Where, O, Observed value; E, expected value; \( \sum \) summation.

DISCUSSION

Detection of Cry1Ab with Bt strips

The genetic segregation and pattern of inheritance of Cry1Ab gene in the genetically modified cowpea were monitored in six crosses of cowpea involving transgenic and non-transgenic lines. In the present study, the segre-
vation of Cry1Ab gene was found to be in Mendelian fashion in all the six cowpea crosses, the results indicate that the resistant trait was controlled by a single dominant gene in the crosses that were examined. The transgenic lines carried the dominant gene while the recessive allele resides in the susceptible genotypes. In the F1 generation studies, the Cry1Ab gene was found to be successfully transferred from transgenic to non-transgenic and it was dominant. These results are in agreement with earlier research works on genetically modified Bt crops with Cry1Ab transgene: Cry1Ab transgene is inherited as single dominant gene, in Bt corn where the Cry1Ab conferred resistance to stem borer (Ostrinia nubilalis) (Murenga et al., 2012), in Bt Rice containing resistant gene to striped stem borer (Chilo suppressalis) (Kiani et al., 2009; Wang et al., 2012), in crosses of transgenic Rojolele Rice (Sulistyowati et al., 2008) and in Bt Cotton where Khan (2008) and Zhang et al. (2008) studied the inheritance and segregation of foreign Bt (Bacillus thuringiensis) toxin and tflA genes. The ability to obtain 3:1 segregation in F2 generations using the Cry1Ab Bt strips means that these tests could be employed for wide-scale studies in the field to enhance cowpea breeding for resistance to M. vitrata.

The results obtained here indicate that it is possible to use this technology to select for Maruca resistant genotypes in cowpea. Similar results have been reported in other crops (corn, soybean, cotton and canola) using Bt strips technology to select plants carrying Cry1Ab transgene (Stave, 2002; USDA/GIPSA 2006) and had proven to be effective in detecting the presence of the transgene in these crops. Cry1Ab Bt strip tests for genetically engineered crops are currently being used on a large scale in the United States to manage the sale and distribution of grains that are genetically transformed (Stave, 2002). In several of these applications, it is important to get a result rapidly in the field, and in these situations strip tests are particularly useful.

Using the Cry1Ab Bt strips, the screening were done at seedling stage with good precision, this saves time and resources. The use of Cry1Ab Bt strips as a screening tool of transgenic lines containing Cry1Ab gene is strongly recommended, this has an important in the hybridization programme where genotypes having the transgene can be distinguished at seedling stage at lesser time. The benefits of this technology have important implications for improving the efficiency of the characterization of cowpea genotypes for resistance to Maruca in the laboratory, especially when working in remote areas and in developing countries where access to laboratory facilities, chemicals, and equipment for polymerase chain reaction (PCR) procedures are limiting. The Cry1Ab Bt strip test was found to be the most suitable in order to rapidly analyze large number of plants in lesser time and to differentiate between the two groups. Elite and promising plants can be faithfully screened and selected at seedling stage particularly during the development of backcross population, aimed towards development of transgenic cowpea varieties. Results obtained from Bt strips sampled materials were effective and reproducible in our hands from the six F2 populations used. The studies described here that the Bt strips screening offering a simple, sensitive and specific tool appropriate for identifying Maruca resistant transgene. We conclude that the application of this technology has the potential to significantly enhance the Maruca resistant cowpea breeding program, and the efficiency of breeders to speed-up the process of developing and deploying Maruca resistant cowpea varieties to farmers. This study demonstrates that Bt strip is an effective, economic and sensitive method for sampling and identifying resistant cowpea plants using leaf tissues.

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