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Research Article

Genetic diversity analysis in Bt introgressed and non-Bt lines of upland cotton (Gossypium hirsutum L.)

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Abstract
Diversity analysis of Bt lines, a prerequisite to heterosis breeding, are rarely published. Diversity analysis of 140 cotton genotypes comprising of BGI, BGII and Non-Bt lines resulted in the grouping of genotypes into 20 clusters among which five had multiple genotypes and 15 others were solitary. Specific clustering of genotypes of BGI and BGII was not observed which got distributed across the clusters. The maximum and minimum inter-cluster distances were observed between two solitary clusters XVI and XVIII (159.8) as well as clusters VII and XI (6.4), respectively, reflecting diverse and close relationship among the Bt genotypes of those clusters. Two pairs of isogenic lines were located in the same clusters as they shared the same genetic background. The genetic background played important role in the diversity rather than the Bt status of genotype. Also, the maximum genetic diversity (64%) was arising from traits which were not related to Bt genes such as plant height, boll weight, and ginning out-turn when compared to the characters related to Bt genes such as seed cotton yield and boll number (33%). In general, for seed cotton yield, BGI outperformed BGII lines. The study helped in developing high yielding hybrids through diverse parental line selections.

Key words: Bt cotton, Genetic diversity, Introgression, D2 analysis

INTRODUCTION
Bt cotton is the most widely adopted genetically modified technology since 1996 when the first Bt cotton variety was approved for cultivation in the USA. By 2021, almost about 80-85% of the global cotton is under transgenic cotton cultivation, predominately with two events BGI (MON 531) and BGII (MON 15985). The rapid adoption of this technology is attributed to its undoubted safety and enormous benefit to farmers and the environment which is reflected in higher yield and less cost by protecting the crop from the damage caused by the bollworms without the need for the costly and environmentally hazardous chemical control. In India, the adoption of Bt cotton hybrids by the farmers have replaced the non-Bt cotton and Bt cotton hybrids that cover 98% of cultivated land. With this expansion of the technology, the hybrid development efforts have increased in Bt cotton.

Genetic diversity is the foundation for the development of new varieties/ hybrids. The precise information about the degree of relationship between different genotypes is very much essential for selecting diverse parents for hybridization programs. Genetic diversity between populations indicates the differences in gene frequencies. The cross involving genetically diverse parents is more likely to produce high heterotic effects as compared with lines that are more closely related to each other.
Genetic diversity analysis in Bt introgressed

Multivariate analysis of the important yield-related characters and the genetic distances of the genotypes involved in the experiments provide valuable hints on possible genetic improvement schemes that can enhance the genetic worth of the population. Mahalanobis $D^2$ statistics has been utilized by several workers in a wide range of crop species including cotton to measure the genetic distance among their breeding material.

Cotton breeding programs across India are increasingly developing Bt cotton purelines to develop hybrids. Genetic diversity studies using Bt genotypes are rarely published, whereas, most of the published reports used non-Bt cotton (Malathi and Patil, 2019; and Nishanth et al., 2015). The current study is aimed to assess the genetic diversity of cotton genotypes consisting of BGII, BGI and Non-Bt lines with estimations of plant characteristics for yield and its contributing characters.

MATERIALS AND METHODS

A set of diverse 140 cotton genotypes comprising of 72 BGII, 45 BGI and 23 Non-Bt genotypes developed by Indo-American Hybrid Seeds (I) Pvt. Ltd. (IAHS) were used to assess the genetic divergence among them. These materials were developed mostly by forward breeding methods using Bt cotton donor line (BGII) crossed with Non-Bt lines selected for desirable segregant that has Bt gene, to isolate Bt cotton pure lines and on a few occasions, isogenic lines were developed by backcross breeding methods. The experiment was conducted at Dharwad, Karnataka, India during the rainy season of 2018 in a randomized complete block design. Two rows of 10 dibbles with a spacing of 90 x 90 cm were planted with each genotype. Observations were recorded in five randomly selected plants in each genotype in each replication on seed cotton yield per plant, boll weight (g), ginning outturn, seed index (g), number of bolls per plant, plant height (cm), number of sympodia and monopodia per plant. The replicated data recorded was used for analysis using Mahalanobis (1936) $D^2$ statistics. Based on $D^2$ values the genotypes were grouped into different clusters by employing Tocher’s method as outlined by Rao (1952).

Table 1. Clustering of genotypes by Mahalanobis $D^2$ analysis

| Cluster | Genotypes | Number of genotypes |
|---------|------------|---------------------|
| I       | ICL-35, ICL-103, ICL-14, ICL-20, ICL-24, ICL-48, ICL-21, ICL-4, ICL-60, ICL-47, ICL-13, ICL-16, ICL-29, ICL-34, ICL-18, ICL-92, ICL-91, ICL-95, ICL-82, ICL-52, ICL-81, ICL-31, ICL-94, ICL-33, ICL-97, ICL-113, ICL-88, ICL-86, ICL-107, ICL-19, ICL-134, ICL-104, ICL-129, ICL-57, ICL-17, ICL-44, ICL-127, ICL-50, ICL-76, ICL-40, ICL-89 | 41 |
| II      | ICL-78 BGI | 1                   |
| III     | ICL-39 BGI | 1                   |
| IV      | ICL-65, ICL-137, ICL-110, ICL-109, ICL-9, ICL-119, ICL-111, ICL-126, ICL-87, ICL-26, ICL-100, ICL-41, ICL-68, ICL-80, ICL-36, ICL-98, ICL-79, ICL-49, ICL-10, ICL-132, ICL-45, ICL-63, ICL-99, ICL-6, ICL-112 | 25 |
| V       | ICL-74, ICL-75, ICL-5, ICL-15, ICL-77, ICL-85, ICL-101, ICL-93, ICL-105, ICL-30, ICL-12 | 11 |
| VI      | ICL-70, ICL-71, ICL-61, ICL-67, ICL-73, ICL-69, ICL-131, ICL-28, ICL-66, ICL-139, ICL-54, ICL-37, ICL-11, ICL-124, ICL-83, ICL-115, ICL-116, ICL-84, ICL-140, ICL-117, ICL-38, ICL-130, ICL-46, ICL-120, ICL-53, ICL-22, ICL-1, ICL-72, ICL-133, ICL-118 | 31 |
| VII     | ICL-23 BGII | 1                   |
| VIII    | ICL-55, ICL-123, ICL-59, ICL-32, ICL-6 , ICL-43, ICL-2, ICL-128, ICL-64, ICL-42, ICL-122, ICL-121, ICL-102, ICL-8, ICL-125, ICL-27, ICL-62 | 17 |
| IX      | ICL-51 BGII | 1                   |
| X       | ICL-7 BGI | 1                   |
| XI      | ICL-90 BGI | 1                   |
| XII     | ICL-25 BGII | 1                   |
| XIII    | ICL-138 NBT | 1                   |
| XIV     | ICL-136 NBT | 1                   |
| XV      | ICL-3 BGII | 1                   |
| XVI     | ICL-135 NBT | 1                   |
| XVII    | ICL-108 BGI | 1                   |
| XVIII   | ICL-58 BGII | 1                   |
| XIX     | ICL-114 BGI | 1                   |
| XX      | ICL-96 BGI | 1                   |

Genotypes with ICL-1 to 72 were BGII, ICL-73 to 117 were BGI and the rest were Non-Bt lines.

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RESULTS AND DISCUSSION

Significant mean squares due to genotypes were observed for all the characters studied indicating diversity in the plant material under investigation. All the 140 cotton genotypes were grouped in twenty distinct clusters based on yield and yield-related traits (Table 1). Among 20 clusters, 15 were solitary clusters and the rest of the clusters were having multiple genotypes. Cluster I contained the maximum number of genotypes i.e. 41 genotypes followed by cluster VI (31 genotypes), cluster IV (25 genotypes), cluster VIII (17 genotypes), and cluster V (11 genotypes). Clusters II, III and clusters IX to XX contained solitary genotypes. The majority of solitary clusters were Bt cotton lines (7 BGII lines and 5 BGI lines). A greater number of genotypes in a single cluster manifested that these genotypes were more closely related and had less genetic variation among them. The genotypes with shared pedigree were mostly clustered in the same group whereas the genotypes with different pedigree had enhanced diversity by being in different clusters. Mugheri (2015) reported a higher number of clusters while studying genetic diversity on Bt-cotton genotypes, indicating the greater genetic diversity for a variety of agronomic and yield traits based on cluster analysis in a group of Bt cotton genotypes. Interestingly, two pairs of isogenic lines (Bt and non-Bt genotypes) were included in the diversity analysis ICL-120 NBt and ICL-1 BGII as well as ICL-131NBt and ICL-54 BGII. All isogenic lines of Bt and non-Bt lines were clustered in the same cluster mainly due to sharing the same genetic background. It further implies that hybridization programs involving genotypes from the same cluster will be of little use in cotton improvement (Sharma et al., 2016). Since the Bt and non-Bt genotypes were found in the same clusters, diversity is more driven by the genetic background than the Bt gene in the genotype. Most of the BGII lines (23 of 75) and BGI lines (15 of 45) were clustered in cluster I (23 lines BGII, 15 BGI and 3 Non-Bt lines). Likewise, in cluster V, seven out of 11 lines were BGI suggesting grouping of genotypes into different clusters did not follow any specific pattern (Sharma et al., 2016). This can be due to the fact that most genotypes are being derived from forward breeding methods of Bt introgression and unidirectional selection.

Table 2. Cluster mean values for nine characters in 140 cotton genotypes.

| Cluster | DFF | PH   | MONO | SYM  | NOB  | BW   | SI    | SCY   | GOT  | Cluster Score | Cluster Rank |
|---------|-----|------|------|------|------|------|-------|-------|------|---------------|--------------|
| I       | 69  | 86.94(17) | 1.4 (6) | 16.9 (15) | 26.8 (12) | 5.1 (9) | 10.4 (12) | 1237 (11) | 35.0 (12) | 98            | 14           |
| II      | 67  | 110.84(7) | 1.5 (5) | 19.2 (3) | 28 (10) | 5.6 (5) | 11.5 (4) | 1260 (10) | 30.1 (18) | 67            | 4            |
| III     | 71  | 90.67 (15) | 1.2 (8) | 15.3 (17) | 31 (7) | 4.9 (11) | 11.3 (5) | 1599 (5) | 30.5 (17) | 97            | 13           |
| IV      | 69  | 100.76 (10) | 1.6 (4) | 17.7 (8) | 32.5 (6) | 4.6 (13) | 9.7 (14) | 1628 (4) | 36.6 (10) | 77            | 10           |
| V       | 69  | 93.06 (13) | 1.6 (4) | 17.1 (13) | 33.5 (5) | 5.3 (7) | 11.0 (7) | 2105 (1) | 36.6 (9) | 67            | 5            |
| VI      | 69  | 87.96 (16) | 1.5 (5) | 17.4 (11) | 27.5 (11) | 3.8 (17) | 9.0 (17) | 930 (17) | 34.8 (13) | 115           | 18           |
| VII     | 71  | 99.5 (11) | 1.2 (8) | 18.3 (6) | 30.2 (8) | 5 (10) | 12.9 (3) | 1192 (13) | 29.2 (19) | 89            | 11           |
| VIII    | 69  | 73.51 (19) | 1.5 (5) | 15.0 (18) | 24.8 (14) | 5.1 (9) | 10.8 (8) | 1188 (14) | 30.9 (16) | 111           | 16           |
| IX      | 66  | 92.34 (14) | 1.5 (5) | 17 (14) | 29.8 (9) | 6.2 (4) | 10.6 (9) | 1398 (6) | 41.8 (1) | 67            | 6            |
| X       | 71  | 113.5 (6) | 1.3 (7) | 17.5 (10) | 21.5 (16) | 4.8 (12) | 10.6 (10) | 1031 (16) | 27.3 (20) | 109           | 15           |
| XI      | 71  | 107.5 (8) | 1.7 (3) | 17.2 (12) | 35.2 (4) | 5.2 (8) | 13.1 (2) | 1315 (7) | 33.2 (15) | 67            | 7            |
| XII     | 70  | 105.67 (9) | 1.7 (3) | 17.9 (9) | 24.8 (13) | 6.3 (3) | 9.4 (15) | 1780 (3) | 38.1 (6) | 70            | 9            |
| XIII    | 67  | 131.5 (2) | 1.5 (5) | 21.2 (2) | 23.8 (15) | 3.9 (16) | 8.8 (18) | 1089 (15) | 35.7 (11) | 90            | 12           |
| XIV     | 65  | 118.5 (5) | 1.6 (4) | 18.5 (5) | 40.5 (3) | 4.6 (13) | 10.5 (11) | 1875 (2) | 41.4 (2) | 48            | 3            |
| XV      | 69  | 121 (4) | 1.8 (2) | 18 (7) | 35.2 (4) | 6.4 (2) | 13.6 (1) | 1314 (8) | 37.7 (8) | 41            | 1            |
| XVI     | 69  | 134.83 (1) | 1 (9) | 21.7 (1) | 42.0 (2) | 4.3 (15) | 9.3 (16) | 1193 (12) | 40.1 (4) | 67            | 8            |
| XVII    | 67  | 126.38 (3) | 1.9 (1) | 19 (4) | 45.7 (1) | 5.5 (6) | 11.3 (6) | 1261 (9) | 37.8 (7) | 42            | 2            |
| XVIII   | 70  | 54 (20) | 1 (9) | 13.8 (20) | 16.2 (18) | 4.5 (14) | 6.8 (20) | 618 (18) | 34.7 (14) | 142           | 20           |
| XIX     | 67  | 95.84 (12) | 1 (9) | 15.8 (16) | 10.8 (19) | 3.4 (18) | 8.7 (19) | 262 (20) | 41.2 (3) | 123           | 19           |
| XX      | 70  | 83.83 (18) | 1 (9) | 14.7 (19) | 17.2 (17) | 6.7 (1) | 10.1 (13) | 548 (19) | 39.8 (5) | 111           | 17           |

DFF- Days to 50% flowering ; PH- Plant height (cm); MONO- Number of monopodia/plant; SYM- Number of sympodia/plant; NOB- Number of boll/plant; BW- Boll weight (g); SCY- Seed cotton Yield (kg/ha); SI- Seed Index (g) ; GOT- Ginning outturn (%) Figures in parenthesis indicates the trait rank

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Analysis of cluster means revealed the relative contribution of different traits to the total divergence by the different clusters. The highest cluster means for the character was given the first rank and the next cluster possessing the next best means were given 2nd, 3rd and so on up to 20th rank for all the traits. Finally, the clusters are ranked based on the overall score obtained from nine characters. The lowest scoring cluster was given the first rank, and the next cluster possessing the score above the previous ones were given 2nd, 3rd and so on up to 20th rank. The utilization of low ranked clusters (high cluster means) in a breeding program is expected to yield desirable lines in the advanced generation of selection (Table 2). In the present investigation, based on morphological and yield contributing characters, it was observed that genotypes grouped under cluster XV were ranked first by having ten characters (1 to 20 scores) in the desirable direction followed by genotypes under cluster XVII with nine characters. Cluster V had the highest mean seed cotton yield and ranked fifth overall which is having Bt cotton lines. Among the top four clusters, three were solitary with Bt genotype. Similar works were reported by Malathi and Patil (2019) and Nishanth et al. (2015).

Intra and inter-cluster distance (D²) values for 140 cotton genotypes grouped into 20 clusters are presented in Table 3. The maximum inter-cluster distance (159.8) was observed between two solitary clusters XVI (Non-Bt) and XVIII (BGII) followed by another pair of the solitary clusters (147.9) between XVII (BG I) and XVIII (BGII). This indicated that the genotypes in these clusters were far diverse than those of other clusters. The cross between the genotypes of these two divergent clusters will give heterotic hybrids with high yield and also the resultant segregants from the crosses would be promising. The minimum inter-cluster distance was observed between clusters VII and XI (6.4) suggesting that genotypes within these clusters were not genetically diverse. The results were in agreement with Akter et al. (2019) and Kavithamani and Amalabalu, (2017). The top three highest intra- cluster distance was observed in cluster VIII (28.8), cluster VI (24.8) and cluster V (19.7). Among these clusters, VIII and V are composed of Bt cotton lines.

The characters contributing maximum to the divergence are given greater emphasis for deciding the cluster for further selection and the choice of parents for hybridization (Siddique et al., 2010). The relative contribution of nine characters towards the total divergence is presented in Table 4. Among nine characters, plant height was the largest contributor (24.38%) towards divergence followed by seed cotton yield (22.27%) and boll weight (17.97%).

| Cluster | I  | II | III | IV | V  | VI | VII | VIII | IX | X  | XI | XII | XIII | XIV | XV | XVI | XVII | XVIII | XIX | XX |
|---------|----|----|-----|----|----|----|-----|------|----|----|----|-----|------|-----|----|-----|-------|-------|-----|-----|
|         | 13.9 | 21.8 | 19.7 | 25.3 | 32.9 | 28.1 | 25.6 | 27 | 18.2 | 28.8 | 29.6 | 30.8 | 56.2 | 54.6 | 40.3 | 74.2 | 53.0 | 46.6 | 69.3 | 38.0 |
| II      | 0  | 15.2 | 26.1 | 34.3 | 34.3 | 41 | 11.3 | 38.3 | 27.7 | 7.86 | 28.1 | 38.5 | 44 | 16.3 | 54.8 | 28.0 | 88.7 | 92.4 | 56.0 |
| III     | 0  | 22.1 | 19.9 | 38.7 | 7.98 | 28.1 | 33.2 | 19.2 | 12.6 | 39.7 | 62.4 | 43.6 | 32.2 | 72 | 44.0 | 84.3 | 105 | 73.0 |
| IV      | 17.2 | 25.5 | 35.3 | 34.4 | 50 | 24 | 33 | 29.8 | 28.9 | 37.5 | 24.7 | 37.1 | 44.3 | 36.0 | 82.6 | 78.3 | 69.0 |
| V       | 19.7 | 56.9 | 37.8 | 51 | 31.3 | 48 | 33.4 | 31.4 | 71.4 | 37.5 | 42.8 | 76.2 | 53.0 | 102 | 123 | 86.0 |
| VI      | 24.8 | 41.3 | 45.1 | 39.2 | 41 | 46.4 | 60.2 | 48.5 | 59.8 | 64.8 | 67.6 | 64.0 | 49.9 | 48.7 | 59.0 |
| VII     | 0  | 32.7 | 44.7 | 15.5 | 6.43 | 58.2 | 62.2 | 56.1 | 25.9 | 72.8 | 42.0 | 96.5 | 102 | 76.0 |
| VIII    | 28.8 | 43.5 | 44.2 | 44.4 | 59.7 | 96.7 | 94.4 | 66.4 | 123 | 88.0 | 51.1 | 100 | 56.0 |
| IX      | 0  | 43.6 | 38.9 | 13.7 | 53 | 39.2 | 29.9 | 59.1 | 39.0 | 57.1 | 69.9 | 24.0 |
| X       | 0  | 23.3 | 39.4 | 31.9 | 56.2 | 32.6 | 59.9 | 43.0 | 89.4 | 78.1 | 62.0 |
| XI      | 0  | 52.4 | 57.1 | 37.6 | 11 | 55.6 | 24.0 | 114 | 105 | 80.0 |
| XII     | 0  | 51.1 | 47.4 | 39.4 | 69.6 | 52.0 | 80.1 | 99.2 | 42.0 |
| XIII    | 0  | 31.1 | 52.8 | 21.2 | 41.0 | 118 | 47.4 | 88.0 |
| XIV     | 0  | 33.6 | 16.9 | 20.0 | 146 | 95.7 | 110.0 |
| XV      | 0  | 45.8 | 13.0 | 134 | 110 | 68.0 |
| XVI     | 0  | 18.0 | 160 | 86.7 | 113.0 |
| XVII    | 0  | 148 | 112 | 90.0 |
| XVIII   | 0  | 64.9 | 35.0 |
| XIX     | 0  | 62.0 |
| XX      | 0  | 0 |

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A similar result was reported by Khan et al. (2015) in transgenic cotton. Boll number and the seed cotton yield which were directly related to the \textit{Bt} gene together contributed 33 per cent diversity whereas, plant height, ginning outturn, seed index and boll weight which are not influenced by the \textit{Bt} gene in the genotype together contributed 64 per cent to the diversity. The above results implied that to select genetically diverse parents, selection strategy should be based on the traits like seed cotton yield, plant height and boll weight. De et al. (1988) stated that traits contributing maximum towards the $D_2$ values needed to be given more emphasis for deciding the clusters to be taken for choice of parents for hybridization. The \textit{per se} performance of transgenic and non-transgenic lines was also compared (Table 5) and the mean performance of seed cotton yield and its contributing characters were higher in BG I group as compared to the BGII group. Better bollworm control in \textit{Bt} genotypes leads to greater retention of early-formed bolls which transformed to higher seed cotton yield (Hebbar et al., 2007). In grouping genotypes, transgenic and non-transgenic lines were spread in all clusters. To develop superior parents, understanding the genetic diversity of the genetic materials is the basic step in the breeding program. In the current study, highly significant differences were observed among \textit{Bt} and Non-\textit{Bt} cotton genotypes evaluated for all the nine studied traits. \textit{Bt} cotton lines were significantly higher yielding than non-\textit{Bt} genotypes. Irrespective of \textit{Bt} or non-\textit{Bt}, genotypes were distributed in 20 clusters (5 with multiple genotypes and 15 with solitary) and there were no specific clusters for \textit{Bt} genotypes. This could be because the \textit{Bt} unrelated traits (plant height, ginning outturn and boll weight) together contributed the maximum to the observed diversity. The majority of the solitary clusters were with \textit{Bt} genotypes (7 BG I and 5 BG II) indicating the forward breeding of the \textit{Bt} genotypes has created diversity. The study helped to select the diverse BG I and BGII parents to develop hybrids.

### Table 4. Contribution of traits towards divergence in cotton genotypes

| S. No. | Trait                        | Contribution (%) |
|-------|------------------------------|------------------|
| 1     | Days to 50% flowering       | 0.46             |
| 2     | Plant height                | 24.38            |
| 3     | Number of monopodia         | 0.68             |
| 4     | Number of sympodia          | 0.4              |
| 5     | Number of bolls             | 10.85            |
| 6     | Boll weight                 | 17.97            |
| 7     | Seed Index                  | 6.28             |
| 8     | Seed cotton yield           | 22.27            |
| 9     | Ginning out turn            | 16.19            |

### Table 5. Performance of transgenic and non-transgenic cotton genotypes for yield and its contributing characters.

| Characters                        | Number of lines | Transgenic status |
|-----------------------------------|-----------------|-------------------|
|                                   | BG II           | BG I              | Non-Bt            |
| Mean                             | Mean | Range | S.E | Mean | Range | S.E | Mean | Range | S.E |
| Days to 50% flowering            | 69    | 66-74 | 0.25 | 69    | 66-73 | 0.3 | 68    | 64-72 | 0.38 |
| Plant height (cm)                 | 88.2  | 51.5-121 | 1.64 | 91.51 | 65.8-126.4 | 2.02 | 95.6 | 54.7-134.8 | 4.35 |
| Number of monopodia /plant       | 1.5   | 1-2.7 | 0.05 | 1.4   | 1-2.2 | 0.05 | 1.5   | 1-2.5 | 0.08 |
| Number of sympodia /plant        | 17    | 13-22 | 0.22 | 17.19 | 15-20 | 0.22 | 17    | 13-22 | 0.48 |
| Number of bolls / plant          | 28.5  | 11.8-45 | 0.7 | 30.0  | 10.8-45.6 | 1.12 | 19    | 16.5-25 | 1.64 |
| Boll weight (g)                  | 5.1   | 3.04-6.4 | 0.1 | 4.9   | 2.9-6.7 | 0.12 | 4.5   | 3.65-4.9 | 0.14 |
| Seed index (g)                   | 9.8   | 6.8-13.6 | 0.17 | 10.4  | 8.2-13.1 | 0.16 | 10.3 | 8.7-13.9 | 0.29 |
| Seed cotton yield (kg/ha)        | 1293  | 993-2222 | 44.41 | 1399 | 262-2226 | 77.0 | 951 | 502-1075 | 66.26 |
| Ginning out turn (%)             | 34.2  | 22.9-41.7 | 0.45 | 36.1  | 30.1-41.9 | 0.46 | 34.9 | 25.9-39.4 | 0.79 |
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