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Potential of ten wild diploid cotton species for the improvement of fiber fineness of upland cotton through interspecific hybridization

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Cotton is the highest source of natural fiber in textile industry worldwide. With the modern spinning technologies, the demand for cotton fiber with higher quality has increased, making the genetic improvement of fiber quality one of the main challenges for cotton breeders. In cotton breeding, wild species are important source of several desirable genes for genetic improvement of the main cultivated cotton Gossypium hirsutum L (Upland cotton). Besides length and strength, fineness is one of the most important criteria associated with cotton fiber quality. In this study, ten wild diploid species of cotton were investigated for their fiber fineness and potential to improve fiber fineness of G. hirsutum L. The method was measuring of ribbon width after caustic swelling. The results showed the potential of four wild species (G. longicalyx Hutch. & Lee, G. anomalum Wawra & Peyr., G. thurberi Todaro and G. stocksii Mast.) to significantly improve the fiber fineness of upland cotton in a hybrid configuration. Among them, G. longicalyx stood out for its exceptional fiber fineness, and its remarkable impact on reducing the fiber fineness of G. hirsutum L. The wild species highlighted in this study constitute an interesting genetic resource for the development of upland cotton varieties with improved fiber fineness.

Key words: Cotton, fiber fineness, Gossypium spp, hybrid, plant breeding, tetraploid species, wild diploid species.

INTRODUCTION

Cotton fiber is the major commercial product from cotton and the most widely used natural fiber in the world’s textile industry (Ayubov et al., 2018). This important fiber crop belongs to the genus Gossypium which includes 46 diploid (2n = 2x = 26) and 7 tetraploid (2n = 4x = 52) species (Fang et al., 2017). All the diploid Gossypium species originated from a common ancestor and diversified into eight genome groups from A to G, and K (Wu et al., 2018). All tetraploid cotton species are allotetraploid and have a genome designated by AD; they come from a natural hybridization event between an A-genome species and a D-genome species, followed by a doubling of the chromosome number 1 to 2 million years ago (Wendel and Groover, 2015; Fang et al., 2017).

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Among the 53 Gossypium species, only four species including two diploids (G. arboreum L and G. herbaceum L) and two tetraploids (G. hirsutum L and G. barbadense L) are cultivated for their spinnable fibre (Gallagher et al., 2017; Wang et al., 2018; Ijaz et al., 2019). The remaining 46 species are wild.

G. hirsutum L, which is also known as Upland cotton, Long Staple cotton or Mexican cotton, is extensively cultivated due to its wide adaptability to the environment, high production, and better yield potential. It fulfills over 90 % of the output of global cotton fiber yield (Shim et al., 2018; Konan and Mergeai, 2020). G. barbadense L, otherwise known as Sea Island cotton, Pima cotton or Egyptian cotton, is known for excellent fiber quality with long, strong, and fine fibers (Avci et al., 2013). It contributes to 8% of the global cotton production (Shim et al., 2018). The cultivated diploid species provide approximately 2% of the world’s cotton and are cultivated in the more traditional growing areas of India, Pakistan, China, Bangladesh and Iran (Kulkarni et al., 2009, Wendel et al., 2010; Shim et al., 2018).

Based on genetic hybridization properties, Gossypium species are grouped into the primary, secondary and tertiary gene pools. Both the cultivated (G. hirsutum L and G. barbadense L) and wild allotetraploids (G. tomentosum Nuttall ex Seemann, G. mustelinum Miers ex Watt and G. dawinii Watt) comprise the primary gene pool of cotton. The secondary gene pool includes the diploids having the A, B, D and F genomes, whereas the tertiary gene pool is composed of species with C, E, G and K genomes (Campbell et al., 2010).

Previously, cotton breeders primarily emphasized yield and agronomic characteristics, but with the recent development of high-speed spinning technologies, the demand for cotton fiber with higher quality has increased, making the improvement of fiber quality highly crucial in Upland cotton (Islam et al., 2016; Shang et al., 2016; Ayubov et al., 2018). Faced with this existing demand and the dynamics of modern textile industry, the perpetual need of genetic improvement in fiber quality is one of the main challenges for cotton breeders today. Biologically, cotton fibers are single-celled trichomes that grow from the epidermal cell layer of the ovule in a boll (Miao et al., 2017; Ayubov et al., 2018; Ijaz et al., 2019). Besides the length and the strength, the fineness is one of the most important criteria associated to cotton fiber quality (Bradow and Davidonis, 2000; Konan and Mergeai, 2020). The fineness of mature fiber is critical for fiber processing. It influences the fabric lustre, dye appearance, fabric stiffness, spinning performance, and yarn strength (Rodgers and Thibodeaux, 2012). The better the fineness of cotton, the more would be the number of fibers per cross-section. This would result in higher yarn strength, which improves spinning efficiency and yarn evenness (Ahmad et al. 2003; Islam et al., 2016).

Cotton fiber fineness can be expressed as the perimeter, diameter or ribbon width (RW), cross sectional area, and standard fiber weight (Rodgers and Thibodeaux, 2012). The indirect methods used for its measurements are Advanced Fibre Information System (AFIS), Fibre Maturity Tester (FMT), and Near Infrared (NIR) spectroscopy, Vibroscope, High Volume Instrument (HVI) for micronaire etc; the most common direct measurements of fiber fineness include cross-sectional image analysis and ribbon width measurement after caustic swelling (Rodgers and Thibodeaux, 2012). The most effective way to improve cotton fiber fineness is through breeding (Nacoulima and Mergeai, 2014; Islam et al., 2016).

Previous progress in the improvement of fiber quality of upland cotton has been mainly achieved using the genetic diversity present in the primary gene pool of cotton (especially G. barbadense L), but currently, this available diversity has been exhaustively utilized (Gotmare et al., 2000; Ayubov et al., 2018). Accordingly, it has become a necessity to exploit useful genes of wild species from the two other gene pools. Indeed, in cotton breeding, wild species constitute an important resource with several useful traits which can be introgressed into the main cultivated species for improvement (Konan and Mergeai, 2020). The objective of the present study is to detect donor parents for fiber fineness by determining the fiber fineness of a collection of wild diploid species using ribbon width measurement and evaluating their potential to improve fiber fineness of upland cotton through interspecific hybridization.

MATERIALS AND METHODS

Plant material

The plant material included plants from the living cotton collection of the Laboratory of Tropical Agro ecology of Gembloux Agro-Bio Tech (Liège University, Belgium). It was composed of eleven diploid cotton species, their bi-species hybrid with G. hirsutum L, one cultivar of the tetraploid species G. barbadense L, four cultivars of the tetraploid species G. hirsutum L and fifteen second back-cross (BC2) progenies of the HTL tri-species hybrid (G. hirsutum L × G. thurberi Todaro)² × G. longicalyx Hutch. & Lee (Table 1). The crossing scheme used to generate the bi-species hybrid and the BC2 progenies of the HTL tri-species hybrid are presented in Figures 1 and 2, respectively. The crossing procedures used are presented in detail by Konan et al. (2007) and Konan and Mergeai (2020). The plants were maintained in a ventilated greenhouse where the growing conditions during capsule maturation period were 55-60% relative humidity and 35-26°C day-night air temperatures. The plants were grown in 5 L pots filled with a 3:2:1 (v:v:v) sterile mixture of compost, sand and peat. Cotton fibers were harvested at full maturity and used for the analysis of their fineness.

Fiber fineness analysis

Fiber fineness analysis was conducted on all the genotypes studied. For this analysis, the fibers were combed and a tuft of parallel fibers was cut from the seed. Their free points were also cut and the median region was placed on a slide and covered with a cover glass.
Table 1. Presentation of the genotype, genome and status of the plant material used in the study.

| Genotype                    | Genome    | Status (distribution)                        |
|-----------------------------|-----------|----------------------------------------------|
| *G. anomalum* Wawra & Peyr. | B:B₁      | Wild diploid species (Africa)                |
| *G. sturtianum* (R.Br.) J. H. Willis | C₁C₁      | Wild diploid species (Australia)             |
| *G. armourianum* Kearney    | D₂₁D₂₁    | Wild diploid species (America)               |
| *G. harknessii* Brandegee   | D₂₁₂D₂₁₂  | Wild diploid species (America)               |
| *G. aridum* (Rose & Standl.) Skovst. | D₁D₄      | Wild diploid species (America)               |
| *G. raimondii* Ulbr.        | D₂D₅      | Wild diploid species (America)               |
| *G. stocksii* Mast.         | E₁E₁      | Wild diploid species (Arabia)                |
| *G. areysianum* Deflers     | E₁₂E₁₂   | Wild diploid species (Arabia)                |
| *G. thurberi* Todaro        | D₁D₁      | Wild diploid species (America)               |
| *G. longicalyx* Hutch. & Lee | F₁F₁      | Wild diploid species (Africa)                |
| *G. arboretum* L.           | A₁₂A₂     | Cultivated diploid species (Indo-Burma, China and Arab) |
| *G. hirsutum* L. (cv. C2)   | (A₁₁D₁₁D₁₁) | Cultivated tetraploid species (Africa)       |
| *G. hirsutum* L. (cv. NC8)  | (A₁₁D₁₁D₁₁) | Cultivated tetraploid species (Africa)       |
| *G. hirsutum* L. (cv. 98M-2983) | (A₁₁D₁₁D₁₁) | Cultivated tetraploid species (Africa)       |
| *G. hirsutum* L. (cv. 11240-RNR) | (A₁₁D₁₁D₁₁) | Cultivated tetraploid species (Africa)       |
| *G. barbadense* L. (cv. 353) | (A₁₁D₁₁D₁₁) | Cultivated tetraploid species (Africa)       |
| (G. hirsutum cv. C2 × G. arboreum)² | 2(A₁₁D₁₁A₂) | Bi-species hexaploid hybrid (Africa)         |
| (G. hirsutum cv. C2 × G. anomalum)² | 2(A₁₁D₁₁B₁) | Bi-species hexaploid hybrid (Africa)         |
| (G. hirsutum cv. C2 × G. sturtianum)² | 2(A₁₁D₁₁C₁) | Bi-species hexaploid hybrid (Africa)         |
| (G. hirsutum cv. NC8 × G. australi)² | 2(A₁₁D₁₁C₁) | Bi-species hexaploid hybrid (Africa)         |
| (G. hirsutum cv. C2 × G. harknessii)² | 2(A₁₁D₁₁D₁₁) | Bi-species hexaploid hybrid (Africa)         |
| (G. hirsutum cv. NC8 × G. aridum)² | 2(A₁₁D₁₁D₁₁) | Bi-species hexaploid hybrid (Africa)         |
| (G. hirsutum cv. C2 × G. raimondii)² | 2(A₁₁D₁₁D₁₁) | Bi-species hexaploid hybrid (Africa)         |
| (G. hirsutum cv. NC8 × G. stocksii)² | 2(A₁₁D₁₁E₁) | Bi-species hexaploid hybrid (Africa)         |
| (G. hirsutum cv. NC8 × G. areysianum)² | 2(A₁₁D₁₁E₁) | Bi-species hexaploid hybrid (Africa)         |
| (G. hirsutum cv. C2 × G. thurberi)² | 2(A₁₁D₁₁D₁₁) | Bi-species hexaploid hybrid (Africa)         |
| (G. hirsutum cv. C2 × G. longicalyx)² | 2(A₁₁D₁₁D₁₁) | Bi-species hexaploid hybrid (Africa)         |
| (G. hirsutum cv. C2 × G. thurberi)² x G. longicalyx BC2 | A₁F₁D₁D₁ | Tri-species tetraploid hybrid (Australia) |
| (G. hirsutum cv. C2 × G. thurberi)² x G. longicalyx | A₁₁F₁₁D₁₁ | Tri-species tetraploid hybrid (Australia) |

![Diagram of development scheme](image)

**Figure 1.** Development scheme of the bi-species hexaploid hybrids. “X” represents a diploid genome (A, B, C, D, E, F, G or K).
One or two drops of 18% NaOH solution was allowed to penetrate into the fibers by capillarity. The NaOH solution swells the fibers (Figure 3). The diameter of at least 100 fibers was then measured with the software NIS-Elements BR 2.30 (Nikon, Japan) using the Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan) equipped with a digital JVC KY-F 58E camera (JVC, Yokohama, Japan). The ribbon width was determined by dividing the mean of the diameters measured by the 1.3 Summers coefficient (Roehrich, 1947; Nacoulima et al., 2016; Konan and Mergeai, 2020).

**RESULTS AND DISCUSSION**

**Analysis of fiber fineness of studied diploid and tetraploid cotton species**

The results of the analysis of fiber fineness for the studied diploid and tetraploid cotton species are presented in Table 2. The ribbon width of the ten wild diploid species varied from 5.940 µm (G. longicalyx Hutch. & Lee) to 15.533 µm (G. thurberi Todaro), while that of the cultivated species ranged from 17.765 µm (G. hirsutum L cv. C2) to 24.374 µm (G. arboretum L). All the wild diploid species had finer fibers than the cultivated species. Their fibers were even finer than the Sea Island cotton (G. barbadense L), which is known for its fine

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**Statistical analysis**

All the data collected were subjected to the analysis of variance (ANOVA) using the software Statistica 7.1 (Stat Soft, France). The least significant difference (LSD) was used to establish the differences between means at P=0.05.
fibers (Avci et al., 2013; Ijaz et al., 2019). Regarding the LSD grouping, the finest fibers among the studied wild diploid species were given by *G. longicalyx* Hutch. & Lee (5.940 µm) and *G. anomalum* Wawra & Peyr. (6.128 µm), followed by *G. harknessii* Brandegee (7.772 µm) and *G. raimondii* Ulbr. (8.570 µm). The other wild diploid species presented values of ribbon width ranging from 10.907 to 15.533 10 µm. The very low ribbon width exhibited by the African wild diploid species *G. longicalyx* Hutch. & Lee underlines its potential to improve fiber fineness (Demol et al., 1978; Nacoulima et al., 2016; Konan et al., 2020). The results also highlighted another African wild species, *G. anomalum* Wawra & Peyr., which presented good fiber fineness close to that of *G. longicalyx* Hutch. & Lee, with no significant difference. The good fiber fineness of *G. anomalum* Wawra & Peyr. has also been reported by Mehetre (2010). The American wild species *G. harknessii* showed finer fiber than *G. raimondii* Ulbr, but it is rarely cited as a good source of fiber fineness like *G. raimondii* Ulbr (Gotmare et al., 2000; Islam et al., 2016). *G. harknessii* Brandegee is most often cited for its resistance to Verticillium wilt and Fusarium wilt, and as source of cytoplasmic male sterility and fertility restorer (Ano et al., 1982; Gotmare et al., 2000).

Among the cultivated species, the Upland cotton varieties *G. hirsutum* L cv. C2 and *G. hirsutum* L cv. NC8 had the finest fibers with 17.765 and 18.294 µm ribbon width respectively; while *G. barbadense* L presented a ribbon width of 19.117 µm. Although *G. barbadense* L is recognized as having finer fiber than Upland cotton (Avci et al., 2013), the present results showed finer fibers for these two varieties of *G. hirsutum* L. Actually, several varieties of upland cotton resulting from breeding programs for fiber quality have gained in fiber fineness comparable to that of *G. barbadense* L; this is the case for these two varieties of *G. hirsutum* L (cv. C2 and cv. NC8) in the present study.

Of the results presented in Table 2, the cultivated diploid species *G. arboreum* L had the highest ribbon width value. This result showed that not all diploid species produce fine fibers, even if all the other (wild) diploid species studied had finer fibers than the tetraploid cotton studied. It again stresses that wild diploid species can be a source of desirable genes for the genetic improvement of cultivated cotton (Konan and Mergeai, 2020).

### Analysis of fiber fineness of the bi-species hexaploid hybrids

To evaluate the influence of the studied diploid genomes on the fiber fineness of upland cotton, hybrids including each of these genomes and genome of *G. hirsutum* L cv C2 or cv NC8 were examined for their fiber fineness. The results of this analysis are shown in Table 3. The mean values of ribbon width of the different hybrid ranged from 12.526 to 26.072 µm. The bi-species hexaploid hybrid (*G. hirsutum* L cv. C2 × *G. longicalyx* Hutch. & Lee)² showed the finest fibers with a mean value of ribbon width of 12.526 µm. It was followed by (*G. hirsutum* L cv. C2 × *G. anomalum* Wawra&Peyr.)² with an average 15.833 µm of ribbon width, and then (*G. hirsutum* L cv. C2 × *G. thurberi* Todaro)² and (*G. hirsutum* L cv. NC8 × *G. stocksii* Mast.)² with mean value of 16.835 and 16.852 µm of ribbon width, respectively. The highest value of ribbon width

### Table 2. Ribbon width of the diploid and tetraploid cotton species studied.

| Genotype               | Number of fiber analysed | Ribbon width (µm ± standard deviation) | Min   | Max   | LSD grouping |
|------------------------|--------------------------|----------------------------------------|-------|-------|--------------|
| *G. anomalum*          | 104                      | 6.128 ± 0.210                          | 3.738 | 9.138 | A            |
| *G. sturtianum*        | 71                       | 10.907 ± 0.255                         | 6.877 | 18.700| D            |
| *G. armourianum*       | 102                      | 13.967 ± 0.212                         | 7.769 | 20.438| F            |
| *G. harknessii*        | 104                      | 7.772 ± 0.210                          | 4.662 | 12.369| B            |
| *G. aridum*            | 100                      | 11.013 ± 0.215                         | 7.123 | 15.931| D            |
| *G. raimondii*         | 110                      | 8.570 ± 0.205                          | 5.592 | 11.585| C            |
| *G. stocksii*          | 101                      | 11.706 ± 0.213                         | 6.069 | 14.562| E            |
| *G. areysianum*        | 102                      | 13.786 ± 0.212                         | 7.685 | 20.085| F            |
| *G. thurberi*          | 83                       | 15.533 ± 0.235                         | 9.054 | 21.938| G            |
| *G. longicalyx*        | 113                      | 5.940 ± 0.202                          | 4.254 | 8.862 | A            |
| *G. arboreum*          | 107                      | 24.374 ± 0.207                         | 16.338| 37.308| K            |
| *G. hirsutum* (cv. C2) | 107                      | 17.765 ± 0.207                         | 12.092| 24.369| H            |
| *G. hirsutum* (cv. NC8)| 116                      | 18.294 ± 0.199                         | 13.885| 24.169| H            |
| *G. hirsutum* (cv. 98M)| 114                      | 19.445 ± 0.201                         | 13.885| 25.785| I            |
| *G. hirsutum* (cv. 11240)| 112                     | 20.036 ± 0.203                         | 13.423| 25.015| J            |
| *G. barbadense* (cv. 353)| 110                   | 19.117 ± 0.205                         | 12.938| 26.638| I            |
was presented by the bi-species hybrid (G. hirsutum L cv. NC8 × G. austral F.Muell.)². As for the diploid species where G. longicalyx Hutch. & Lee and G. anomalum Wawra & Peyr had the smallest ribbon width, it was the hexaploid hybrids which contained genomes of G. longicalyx Hutch. & Lee or G. anomalum Wawra&Peyr which showed the smallest ribbon width. However, the hexaploid hybrid including G. longicalyx produced significantly finer fibers than the hybrid including G. anomalum Wawra & Peyr. This result indicates the greater impact of the F₁ genome of G. longicalyx Hutch. & Lee in the improvement of fiber fineness of upland cotton than the B₁ genome of G. anomalum Wawra & Peyr. The results also showed that the D₁ genome of G. thurberi Todaroand E₁ genome of G. stocksii Mast.reduced the fiber fineness of G. hirsutum L as well, but not as much as G. longicalyx Hutch. & Lee and G. anomalum Wawra & Peyr.

Apart from the four wild diploid species G. longicalyx Hutch.& Lee, G. anomalum Wawra & Peyr, G. thurberi Todaro and G. stocksii Mast, all the other diploid species did not bring an interesting improvement in fiber fineness of G. hirsutum L. Even some wild diploid species such as G. harknessii Brandegee (genome E3) and G. raimondiiUlbr. (genome D5) which had good fiber fineness (ribbon width <10 µm) could not reduce the ribbon width of G. hirsutum L when combined to it in bi-species hybrids. These results suggest that the genes that control the fineness of the fibers in the different wild diploid species did not have the same action when they are confronted with the genome of upland cotton in a hybrid configuration. The diameter of the cotton fiber is primarily a genetic trait and the genetic mechanisms of fiber traits are complex (Matic-Leigh and Cauthen, 1994; Bradow and Davidson, 2000; Zhang et al., 2013; Islam et al., 2016). According to Ijaz et al. (2019), cotton fiber quality traits are controlled by multiple genes (polygenic inheritance) with different mechanisms and complex genetic architecture. For instance, in the past decades, studies on cotton fiber quality traits on G. hirsutum L and G. barbadense L found a significant association between SSRs and fiber quality traits and identified 70 stable loci for target traits including 30 for fiber length, 27 for fiber strength, and 13 for fiber fineness (Zeng et al., 2009; Cai et al., 2014). Later, several studies, on cotton fiber quality traits that focused on both G. hirsutumL and G. hirsutum L×G. barbadenseL populations, have mapped fiber QTLs in large genomic regions that may include hundreds or thousands of genes (Said et al., 2013; Fang et al., 2014; Shang et al., 2015; Tang et al., 2015; Tan et al., 2015; Ma et al., 2017, 2018; Ijaz et al., 2019). QTLs are chromosomal regions which contribute cumulatively to a trait with varying percentages of phenotypic variance from each QTL (Said et al., 2015). According to Ijaz et al. (2019), the number of fiber quality trait QTLs over the chromosomes of the cotton genome is not identical, and QTLs associated with cotton fiber quality obtained from Cotton QTL database (http://www.cottonqtldb.org) are distributed unevenly across the 26 chromosomes of the cotton genome.

### Analysis of fiber fineness of the tri-species hybrid and its BC2 progenies

The HTL tri-species hybrid (G. hirsutum L × G. thurberi Todaro)² × G. longicalyx Hutch. & Lee (Konan et al., 2007) with a ribbon width of 12.65 µm (Table 3) had the same fiber fineness as G. hirsutum L × G. longicalyx Hutch. & Lee² hexaploid hybrid (P<0.05). To check the behavior of the genes of G. longicalyx Hutch. & Lee responsible for the fiber fineness in the advanced progenies of the tri-species hybrid, HTL/BC2 plants were examined for the fineness of their fibers. The results of

| Genotype                                      | Number of fiber analysed | Ribbon width (µm) ± standard deviation | Min   | Max   | LSD groupings |
|----------------------------------------------|--------------------------|----------------------------------------|-------|-------|---------------|
| (G. hirsutum cv. C2 × G. arboreum)²          | 100                      | 22.306 ±0.199                          | 15.615| 27.646| H             |
| (G. hirsutum cv. C2 × G. anomalum)²         | 110                      | 15.833 ±0.190                          | 11.331| 20.523| B             |
| (G. hirsutum cv. C2 × G. sturtianum)²       | 106                      | 19.499 ±0.193                          | 12.538| 26.338| F             |
| (G. hirsutum cv. NC8 × G. austral)²         | 112                      | 26.072 ±0.188                          | 19.877| 33.046| J             |
| (G. hirsutum cv. C2 × G. harknessii)²      | 116                      | 20.204 ±0.185                          | 14.415| 25.446| G             |
| (G. hirsutum cv. NC8 × G. aridum)²         | 104                      | 18.183 ±0.195                          | 14.462| 21.915| D             |
| (G. hirsutum cv. C2 × G. raimondii)²       | 104                      | 18.853 ±0.195                          | 14.415| 22.077| E             |
| (G. hirsutum cv. NC8 × G. stocksii)²       | 107                      | 16.852 ±0.196                          | 12.069| 20.977| C             |
| (G. hirsutum cv. NC8 × G. areysianum)²      | 117                      | 22.937 ±0.192                          | 16.500| 28.438| I             |
| (G. hirsutum cv. C2 × G. thurberi)²        | 122                      | 16.835 ±0.184                          | 12.215| 23.208| C             |
| (G. hirsutum cv. C2 × G. longicalyx)²      | 120                      | 12.649 ±0.182                          | 10.008| 15.277| A             |
Table 4. Ribbon width of the BC2 progenies of the HTL tri-species hybrid.

| Genotype          | Number of fiber analysed | Ribbon width (µm) ± standard deviation | Min  | Max  | LSD grouping |
|-------------------|--------------------------|----------------------------------------|------|------|--------------|
| HTLBC2#1          | 102                      | 15.332 ±0.171                          | 10.262 | 20.977 | C            |
| HTLBC2#3          | 110                      | 14.906 ±0.165                          | 10.331 | 19.554 | BC           |
| HTLBC2#9          | 110                      | 17.388 ±0.165                          | 10.877 | 21.754 | I            |
| HTLBC2#11         | 102                      | 14.650 ±0.171                          | 11.331 | 18.292 | B            |
| HTLBC2#14         | 101                      | 16.842 ±0.172                          | 13.692 | 20.523 | GH           |
| HTLBC2#15         | 100                      | 16.519 ±0.173                          | 12.008 | 23.838 | FG           |
| HTLBC2#17         | 101                      | 16.931 ±0.172                          | 11.331 | 21.385 | GHI          |
| HTLBC2#18         | 103                      | 15.977 ±0.171                          | 12.538 | 20.415 | DE           |
| HTLBC2#5          | 116                      | 13.922 ±0.161                          | 10.723 | 17.154 | A            |
| HTLBC2#6          | 121                      | 16.356 ±0.157                          | 11.808 | 20.692 | EF           |
| HTLBC2#7          | 111                      | 13.473 ±0.164                          | 8.038  | 16.654 | A            |
| HTLBC2#10         | 112                      | 15.876 ±0.164                          | 12.008 | 22.077 | D            |
| HTLBC2#13         | 122                      | 15.328 ±0.157                          | 9.885  | 20.523 | C            |
| HTLBC2#16         | 117                      | 17.024 ±0.160                          | 13.400 | 20.962 | HI           |
| HTLBC2#20         | 109                      | 16.757 ±0.166                          | 10.238 | 23.154 | FGH          |

Figure 4. Ribbon width (µm) of parental species and the BC1 and BC2 progenies of the HTL tri-species hybrid. Ribbon width values of the BC1 plants come from the study of Konan et al. (2020).

due to the segregation of G. longicalyx alleles among the BC plants. This suggests the differential presence or absence of this diploid species chromosomes and/or chromosome recombinants as shown by Konan and Mergeai (2020) with genomic in situ hybridization (GISH) analysis. The persistence of the outstanding fiber fineness of G. longicalyx Hutch. & Lee, in the bi-species hybrid with G. hirsutum L, in the HTL tri-species hybrid and in the HTL/BC1 and BC2 derivative plants demonstrates the inheritance of this trait through the crossing scheme (Figure 4). Hence, this finding brings out the good donor status of G. longicalyx Hutch. & Lee
for fiber fineness. In addition, according to Demol et al. (1978), the fibers of *G. longicalyx* Hutch. & Lee have exceptional fiber strength and a high molecular weight. Such finer and stronger fibers than those of *G. barbadense* L would undoubtedly be much appreciated by spinners. These results therefore make *G. longicalyx* Hutch. & Lee an interesting source that deserves more attention from breeders for the improvement of cotton fiber quality.

**Conclusion**

The results obtained in the present study show the potential of four wild cotton diploid species (*G. longicalyx* Hutch. & Lee, *G. anomalum* Wawra & Peyr., *G. thurberi* Todaro and *G. stocksii* Mast.) to significantly improve the fineness of the fibers of upland cotton in a hybrid configuration. However, among these wild species, *G. longicalyx* Hutch. & Lee stood out for its exceptional fiber fineness, and its remarkable impact on improving the fiber fineness of *G. hirsutum* L. This wild African diploid species seems to be a good donor for the introgression of this useful trait into upland cotton. In view of the results of this study, the species *G. longicalyx*, and to a lesser extent the three other highlighted wild species, constitute interesting genetic resources for the development of cotton varieties with outstanding fiber fineness.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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