Introgression in tetraploid cotton of the resistance to reniform nematode *Rotylenchulus reniformis* Lind. & Oliveira from the *Gossypium hirsutum* L. x *G. longicalyx* Hutch & Lee x *G. thurberi* Tod trispecific hybrid.

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Summary

In order to develop upland cotton plants resistant to Rotylenchulus reniformis *Lind. & Oliveira reniform nematode, a three-species hybrids including* Gossypium hirsutum *L. (2n = 4 x = 52, $(AD)_1$ genome) was created using* G. longicalyx *Hutch & Lee as donor parent (2n = 2x = 26, F_1 genome) and* G. thurberi *Tod. (2n = 2x = 26, D_1 genome) as bridge species. The morphology and the resistance to reniform nematode of the parents and of thirty plants belonging to the selfed progeny of* G. hirsutum *x* G. longicalyx *x* G. thurberi *(HLT) trispecific hybrid were assessed.* G. longicalyx, G. hirsutum *x* G. longicalyx *hexaploid and all the thirty plants issued from the selfing of HLT hybrid were very resistant to reniform nematode unlike* G. hirsutum *which was very sensitive to it. Cytogenetic studies showed that HLT hybrid and its direct progeny have 52 chromosomes as the main cotton cultivated species. HLT hybrid shows a good pollen fertility and a very high level of chromosome pairing at metaphase I. It is self fertile but presents important incompatibility barriers when crossed as female parent with* G. hirsutum*. The prospects to develop upland cotton commercial cultivars resistant to reniform nematodes from the available interspecific genetic stocks are discussed.*

Introduction

The reniform nematode *Rotylenchulus reniformis* Linford and Oliveira 1940 is widely distributed in many tropical and subtropical regions and in warmer parts of the temperate zone of the world (Ayala and Ramirez, 1964; Robinson, 1999; Ferris, 2002). It has been reported in tropical and sub-tropical West and Central Africa; Central and south America; Southeast Asia, the Carribean, Mexico, Japan, the Middle East, South Pacific, Italy, Spain, China and the Far East. *R. reniformis* is present in most cotton-producing areas causing serious problems of production (Robinson, 1999). The deleterious effects of this parasite on cotton are, on the one hand, delay of plant growth and development, and, on the other hand, reduction in lint yield, boll size, lint percent, seed index, and fiber micronaire value (Cook and *al*., 1997; Robinson, 1999). In addition, the reniform nematode is important for increasing the incidence and severity of *Fusarium* wilt on cotton (Kinloch and Rich, 2001). In the regions infested by this nematode, cotton losses goes from 15 to 75% according to the levels of infestation and the meteorological conditions (Yik and Birchfield, 1984; Robinson, 1999; McLean and Weaver, 2002). The most usual strategies for managing *R. reniformis* in cotton include nematicide applications and rotation with nonhost crops. Nevertheless, *R. reniformis* has a very wide host range (Ajala and Ramirez, 1964), rendering often crop

rotation an ineffective method of control. The use of nematicides is uneconomical and for various reasons not always efficient and sometimes dangerous for environment. Therefore, growing nematode-resistant cultivars of cotton appears to be the most economic method of management and the most respectful of environment. Unfortunately, for the time being, no cotton cultivars with a good resistance to *R. reniformis* is available (Robinson and *al*., 1999; Kinloch and Rich, 2001; McLean and Weaver, 2002). Among the 46 diploid species of the genus *Gossypium*, there is however one, *G. longicalyx* Hutch. and Lee (F genome), that seems to be immune to this nematode (Yik and Birchfield, 1984). The introgression of this useful trait from *G. longicalyx* into the main cultivated tetraploid cotton *G. hirsutum* L. (genome A_hD_h) is the main objective of our work. We present here, the results we have obtained regarding the development of fertile tetraploid cotton genotypes resistant to *R. reniformis*.

Materials and Methods

Production of plant materiel, and morphological and cytological observations

According to the pseudophyletic introgression method (Mergeai, see these proceedings), the hexaploid *G. hirsutum* x *G. longicalyx* was crossed to *G. thurberi* to create the trispecific hybrid *G. hirsutum* x *G. longicalyx* x *G. thurberi* (HLT). This hybrid was selfpollinated and backcrossed to *G. hirsutum* (figure 1). Morphological observations were made on the HLT hybrid and on its selfed progeny. In order to determinate chromosomes number and association, flower buds of HLT and its selfed progeny were used to perform cytological analysis. They were fixed in Carnoy's solution (95% ethanol-chloroform-glacial acetic acid, 6:3:1 v:v:v) for 72 h and stored at 4 °C in 70 % ethanol until their evaluation. On a slide, microsporocytes were squashed and stained in a drop of 1.5% acetocarmine solution enriched in iron. The slide recovered with a cover slip was heated a few seconds on a flame to improve chromosomes coloration. After being well flattened, cells were examined for meiotic configurations with a microscope Nikon Eclipse E800 equiped with a JVC KY-F 58E camera.

Pollen fertility was also determined. For each plant, about 1000 pollen grains were dipped in 1.5% acetocarmine solution for 30 min and, pollen fertility was estimated under a stereomicroscope as the percentage of stained pollen; only large, bright and red grains were considered fertile.

Resistance to R. reniformis

Resistance to *R. reniformis* of HLT parents (*G. hirsutum*, *G. longicalyx*, *G. thurberi* and the hexaploid *G. hirsutum* x *G. longicalyx*) and thirty plants of HLT selfed progeny were assessed in two successive experiments. The first experiment was led in a growth chamber programmed for 12-hour light ($120–140.10^{-6}$ E/m².s) and 12-hour dark with air day and night temperatures of 28 °C and 26 °C respectively. Air humidity was about 55%-60%. The second experiment was carried out during spring and summer in the tropical greenhouses of the Gembloux Agricultural University where growing conditions were not controlled.

Reniform nematode egg inoculations were realised on 30 days plants planted in 5 litres pots filled with a 3:2:1 (v:v:v) mixture of compost, sand and peat. The planting medium in each pot was infested with 6000 *R. reniformis* eggs by injecting the appropriate nematode eggs suspension 2 to 3 cm deep at four points 2 cm from the stem. Sixty days after inoculation, the soil was removed by soaking the roots in water and entire root systems were gently harvested. Roots with nematode egg masses were blotted dry with absorbing paper and weighed. Eggs were extracted with 10 % NaOCl (sodium hypochlorite) followed by centrifugation flotation in kaolin and in $MgSO_4$ solution of 1.18 density. Eggs were counted by using two 15 ml aliquot and the number of eggs per

gram of root was determined for each plant. The host status was assessed according to the scale proposed by Yik and Birchfield (1984) where relative plant resistance is based on egg production per gram of root expressed as a percentage of egg production per gram on *G. hirsutum* control plants within the test. This scale contains the following classes of infestation : 0% = immune, 1-10% = highly resistant, 11-25% = resistant, 26-40% = moderately resistant, 41-60% = low susceptible, 61-100% = susceptible as check, and above 100% = very susceptible.

Results

Production of plant materiel and morphological observations

HLT hybrid has a slender habit and is self fertile. All the capsules harvested from HLT issued from self pollination are smaller than those of *G. hirsutum* and the numbers of seeds they contained ranged from 2 to 8. These seeds carried the same dirty-white lint but presented linter with three types of colour. 0n 74 seeds examined, 40 had green linter, 28 had pale-green linter, and 6 had brown linter. Some seedlings coming from these seeds presented distortions at cotyledon leaves stage. In fact, on 33 seedlings belonging to the HLT selfed progeny, 7 seedlings were distorted. Their cotyledon leaves were welded on the petioles or on the entire length of the leaves giving the impression of an unique leaf. These distortions seem, however, to be minor and they had generally no detrimental consequences on the development of the plants.

HLT selfed progeny plants, as HLT hybrid, presented a slender habit with vegetative branches shorter than 40 cm length. The capsules had relatively small size. Tow types of plants were noticed. Plants carrying rather small embossed leaves and the plants producing large and normal leaves. The plants were 152 to 236 cm tall with 12 to 34 nodes on the main stem. The total fruit bearing nodes ranged from 5 to 19 and time from sowing to flowering varied between 103 and 133 days. All these data were superior to those of the *G. hirsutum* control plants, which were 70 to 91 cm tall with 8 to 12 main stem nodes. The total fruit bearing nodes on the control plants varied from 5 to 10 and the time from sowing to flowering was 64 to 84 days. These results are presented in Table 1. HLT and its selfed progeny seem to be photoperiodic. The flowering of these plants only started in end of September when the day length in Gembloux was close to 12 hours.

Backcrosses of HLT hybrid to *G. hirsutum* gave a very low success rate. On 57 backcrosses performed, only one seed was obtained.

Pollen fertility and, chromosomes number and association

Pollen fertility estimated on *G. hirsutum* and HLT hybrid was respectively 98% and 96% whereas the one observed on about twenty plants of HLT selfed progeny varied from 9.50% to 96.7%. There is a distinct segregation concerning pollen fertility of HLT selfed progeny plants but we noted that more than the half of the analysed plants, presented a pollen fertility superior to 70%.

Chromosome counts showed that both HLT hybrids and its selfed progeny have 52 chromosomes. Univalents, bivalents and multivalents were observed. The frequency of these associations per cell was 2n=4x=52 chromosomes = 0.66I + 24.76II + 0.47III + 0.047IV + 0V + 0.047VI in HLT and 2n=4x=52 chromosomes = 0.85I + 24.29II + 0.62III + 0.038IV + 0.038V + 0.038VI in one plant belonging to HLT selfed progeny (Table 2). These results indicate a high level of pairing similar to the one observed by Brown and Menzel (1950) in the *G. hirsutum* x *G. herbaceum* x *G. armourianum* trispecific hybrid which is consistent with the global good self fertility observed in these materials.

Resistance to R. reniformis

Among the parent genotypes of HLT tested in growth chamber, *G. hirsutum* presented the greatest number of eggs per gram root (14 690 eggs.g^{-1}). *G. thurberi* comes to the second position (8 570 eggs.g^{-1}). The number of eggs per gram root of *G. longicalyx* and the hexaploid were very low (186 and 288 eggs.g^{-1} respectively). In these conditions, *G. thurberi* was low susceptible (58% eggs.g^{-1}) whereas *G. longicalyx* (1.27% eggs.g^{-1}) and the hexaploid (1.96% eggs.g^{-1}) were very resistant. These results are presented in Table 3 .

Concerning the HLT selfed progeny, all the thirty plants tested, were very resistant to *R. reniformis* with a percentage of eggs per gram of root varying between 0 and 9.34% in greenhouse conditions. Table 4 presents these results.

Discussion and conclusion

The pseudophyletic method has permitted to obtain the trispecific HLT hybrid. This hybrid is autofertile with a good level of pollen fertility (96%). Morphological observations made on HLT plants and on its selfed progeny showed some traits that are not desirable for a commercial variety: plants too tall; very long cycle, very small capsules; fibre not completely white and not very abundant. To get rid of all these traits coming from the wild donor and bridge species, a program of backcrosses with the recipient species *G. hirsutum* is essential. The first backcrosses realised with HLT hybrid as female and *G. hirsutum* (var. gazuncho) as male did not give satisfying results (one seed was produced for 57 backcrosses). This very low success rate is probably due to the presence of incompatibility barriers between HLT hybrids and the male parent *G. hirsutum*. Cytogenetic data show 52 chromosomes for both HLT and its selfed progeny as the main cotton cultivated species. They indicate also a low presence of univalents and multivalents and a high number of bivalents (about 24II). This level of pairing is one of the highest observed so far in trispecific synthetic allotetraploid hybrids. It confirms the close "relatedness" of *G. longicalyx* with A-genome species. Results of the evaluation of resistance to *R. reniformis* of the HLT hybrid parents show that *G. hirsutum* is susceptible (100% eggs.g^{-1}) and G *thurberi* is lowly susceptible (58% eggs.g^{-1}) whereas *G. longicalyx* (1.27% eggs.g^{-1}) and the hexaploid (1.96% eggs.g^{-1}) are very resistant. These results confirm the high resistance of *G. longicalyx* to reniform nematode *R. reniformis*. They also reveal the expression of this trait in the hexaploid (*G. hirsutum* x *G. longicalyx*)^2. The assessment of 30 plants belonging to the HLT selfed progeny revealed the high level of resistance to *R. reniformis* of all of them. There was practically no segregation between them for this trait; which contrasted with the segregation noticed for the morphological traits. With their high rates of chromosomes pairing, their good level of auto fertility, and their high resistance to reniform nematodes, HLT S_0 and HLT S_1 seem to be the ideal candidates to obtain the introgression of the desired immunity to reniform nematodes in upland cotton. To reach this goal, it will be however necessary to overcome the incompatibility barriers that exists between the trispecific hybrid and *G. hirsutum*.

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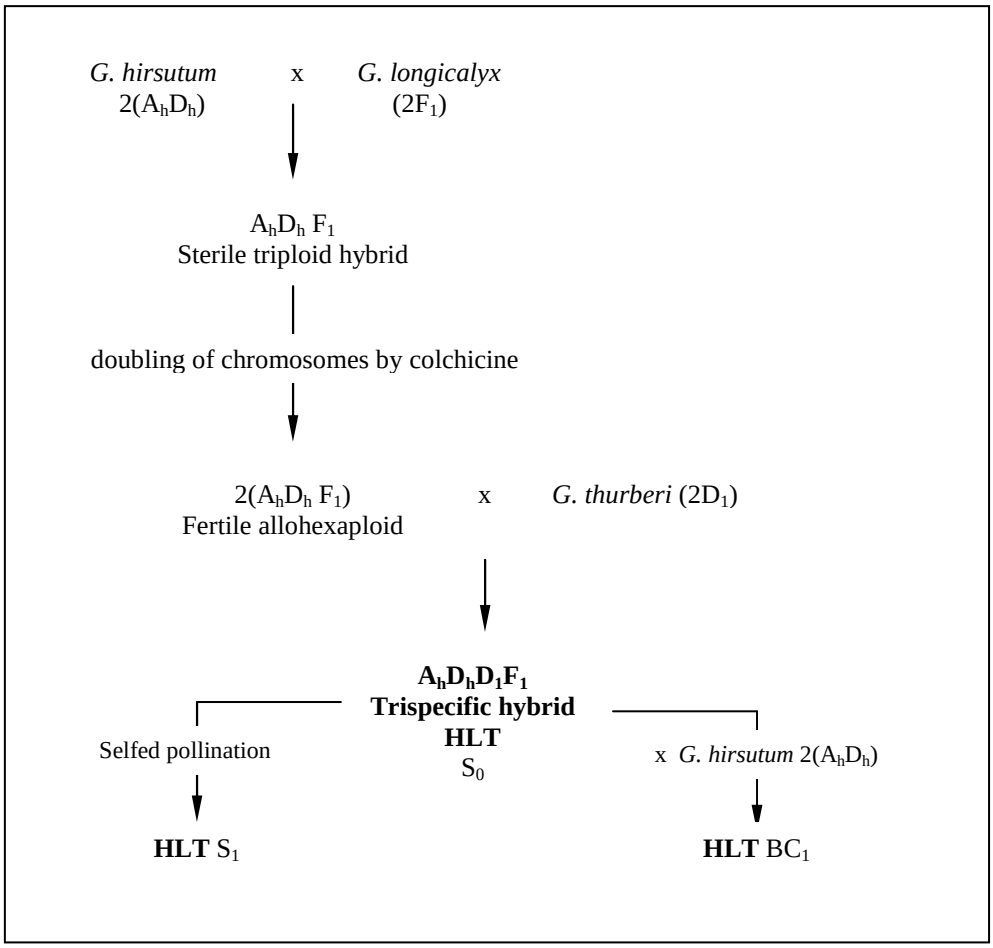

Figure 1. – Development and exploitation scheme of HLT (*G. hirsutum x G. longicalyx x G. thurberi*) trispecific hybrid.

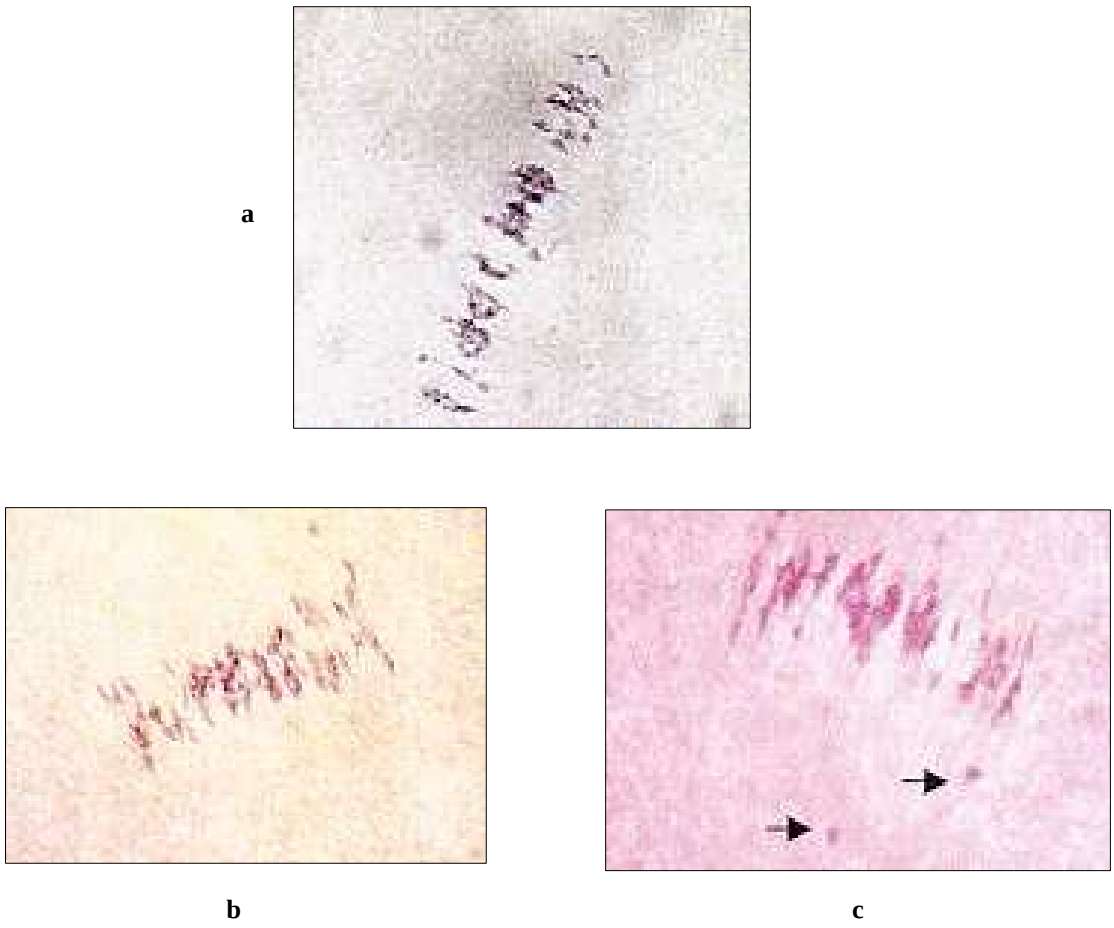

Figure 2. – Chromosomal configuration at metaphase I of meiosis: a) cell of HLT presenting 26 bivalents; b) 26 bivalents in a cell of HLT self progeny; c) 2 univalents (arrow) and 25 bivalents in a cell of HLT self progeny.

Table 1. Descriptive parameters of HLT selfed progeny plants

Genotype	Descriptive parameters	Flowering cycle (days)	Main stem nodes	Fruitbearing nodes	Plant height (cm)
25 plants of HLT selfed progeny	Average	116	25	13	192
	Min - Max	103-133	12-34	5-19	152-236
	Standard deviation	7.91	5.11	3.80	23.49
	variance	59.99	25.04	13.83	528.78
5 control *G. hirsutum* plants	Average	76	11	9	82
	Min - Max	64-84	8-12	5-10	70-91
	Standard deviation	8.50	1.52	2.07	8.35
	variance	57.84	1.84	3.44	55.84

Table 2. Result of cytogenetic analysis on HLT hybrid and its selfed progeny.

Genotype	Descriptive parameters	Chromosome configuration						No of chromosomes	No of cells observed
		I	II	III	IV	V	VI		
HLT	Mean number per plate	0.66	24.76	0.47	0.047	0	0.047	52	27
	Max – Min	0 - 4	20 - 26	0 - 2	0 - 1	0 - 0	0 - 1		
HLT selfed progeny	Mean number per plate	0.85	24.29	0.62	0.038	0.038	0.038	52	21
	Max – Min	0 -3	18 - 26	0 - 4	0 - 1	0 - 1	0 - 1		

Table 3. Results of the assessment in growth chamber of the resistance to *Rotylenchulus reniformis* of parent genotypes of the HLT hybrid .

Genotypes	eggs produced per gram roots	percentage of egg productions per gram root	Host status
G. hirsutum (C2)	14 690	100 %	S*
G. thurberi	8 570	58 %	LS**
G. longicalyx	186	1.27 %	HR***
Hexaploid	288	1.96 %	HR

* S= susceptible; ** LS: low susceptible; ***HR: highly resistant

Table 4. Results of the evaluation in greenhouse of the resistance to *R. reniformis* of 30 plants belonging to the HLT selfed progeny .

Descriptive parameters	Eggs produced per plant	Percentage of egg produced per gram of root	Host status
Max - min	0 – 1 334	0 – 9.34*	
Standard deviation	336	2.4	Highly resistant
Variance	109,117	5.57	

* Data calculated considering a mean infestation in cotton control plants of 207 eggs per gram of root