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Study of Germination and Health Quality of Cotton Seed Grown in the West Central Region of Côte d'Ivoire

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Original Research Article

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Abstract: In Côte d'Ivoire, cotton cultivation faces many constraints including the thorny issue of quality seeds for meeting the challenge of optimal yield. In order to contribute to the achievement of this goal, we investigated the health and germination of cotton varieties used in west central region of Côte d'Ivoire. The cotton seed samples were collected from 10 farmers. Samples comprised 3 varieties which were grouped for laboratory and field analyses including Purity analysis, moisture content test, germination test and the seed borne mycoflora investigation. The result show maximum pure seed (95%) was found in cotton seed samples with a moisture content of 10 %. Cotton seeds of the 3 varieties had very low germination (<30%), highest number of dead seed (27% - 95%) and abnormal seedlings (1 - 51 %) in the laboratory and in the experimental plot. Thereafter, seeds treatment with Thiram + Imidacloprid helped raise the germination rate to more than 70%. Six fungal genera were associated with the 3 cotton seed samples. The genera were Aspergillus, Fusarium. Curvularia, Rhizophus, macrophomina and Paecilomyces. These fungi might be responsible for the deterioration in seed affecting germination and could be involved in seedling blight observed on the experimental field. Using certified seed free from pathogens and disease is essential for successful cotton cultivation in Côte d'Ivoire.
Keywords: seed quality, pure seed, mycoflora, seedling blight, certified seed.

INTRODUCTION

Cotton (Gossypium hirsutum L.) plays an important role in the agricultural economy of Côte d'Ivoire. It ranks third in exports after cocoa and coffee.

Until 2001, cotton was the backbone of the rural economy of northern Côte d'Ivoire, and provided direct livelihood for 180 000 producers, that is, about 2.5 million people [1]. This influence is largely due to research that has developed cropping systems so as to increase cotton yield [2]. But since 2003 the cotton sector has been shaken by the current economic context marked by the upward trend in the prices of chemical inputs and the instability of the purchase price of seed cotton on the national and world market [3, 4]. To this crisis must be added the great inter and intra-annual variability of rainfall, the depletion of soils, the poor application of crop management techniques, the new outbreak of parasitism and the use of non-certified seeds of various origins [5]. This has led to a decline in cotton yield in the North to the detriment of food crops that contribute to improving the socio-economic conditions of farmers [4].

Indeed, cotton cultivation is subject to a full array of pests. They cause a significant decline in yield in a proportion of 50 to 75% [6, 7]. In association with or independently of damage caused by pests, crop losses due to disease are considered to be the most important causes of reduced quantity and quality of products [8]. The major diseases of cotton plant for which the national average risk of yield loss may be highest are Fusarium wilt, bacterial blight, and biologically transmitted diseases such as virosis and cotton virescence [8, 9]. Most of the pathogens responsible are seed borne pathogens that might reduce the seed vigour and weaken the plant at the initial of its growth [8, 10]. These pathogens can be mixed in seed lots, on impurities, on seed surface, in their embryo or their integuments [11]. The use of infected seeds therefore reduces crop potential yield and can also contaminate cropping areas so far uninfected [12]. Therefore, disease control must be both temporal and spatial. The control must therefore take place at three

levels, namely preventing risk appearance, preventing risk occurrence or curbing the appearance of damages [13]. The use of resistant varieties and/or best quality seed is decisive for assessing the reduction of the quantity of inocula accessible by the cropping systems adopted.

In Côte d'Ivoire, important research on cotton protection has been carried out in entomology field [14-17]. In contrast, many efforts remain to be made in pathology where cotton plant is faced with several bacterial and cryptogamic diseases mainly cause by seed-borne pathogens [10, 8].

The main objective of the present study was to evaluate the health and germination qualities of cotton seeds used in West Central region in Côte d'Ivoire so as to contribute to the local management of cotton phytosanitary risks exacerbated by climate change in Côte d'Ivoire.

MATERIALS AND METHODS

Plant material and seed collecting conditions

The plant material consisted of cotton seeds (Gossipium hirsutum L.) and plant organs derived from seedlings stemming from such seeds. Seed collection was made from 10 cotton producers in West Central Côte d'Ivoire during sowing periods from June to July (Table 1). A preliminary survey in West Central cotton fields was carried out and made it possible to select easily accessible rural farms. In the end, 10 producers were selected on the Daloa-Séguéla road. Seed collection was done directly from them during the crop year 2016. The weight of seed samples collected varied between 250 g and 500 g per sample. The different samples were grouped per variety and kept in a cold chamber for study.

Table-1: Localities and varieties of cotton grown in 10 fields in the West Central Region

Sample	Localities	varieties	Date of collection
P1	Worofla	Y301AR3	7 June
P2	Somana1	Y764AR3	8 June
P3	Sifié1	Y764AR3	13 June
P4	Somana2	Y301AR3	15 June
P5	Séguéla1	Y331BR4	18 June
P6	Séguéla2	Y301AR3	22 June
P7	Séguéla3	Y764AR3	26 June
P8	Sifié2	Y764AR3	8 July
P9	Mankono	Y764AR3	18 July
P10	Vavoua	Y301AR3	14 June

Purity and moisture content analysis

For Purity test conducting a sub-sample of 100 g of each variety was manually and visually sorted out into 3 components. These included intact seeds of the corresponding variety (pure seeds), other crop seed mixed in the sample and some inert matter. The results obtained were expressed as a percentage of pure seeds and impurities. Pure seeds were tested for seed moisture content, germination and seed health.

The seed moisture content was done by drying seed in an oven for 17 hours at 103 °C. In that respect, 100 g of seeds were weighed before (M1) and after (M2) passing through the oven. The moisture content (TE) was determined according to the following formula:

$$TE(\%) = \frac{M1 - M2}{M1} X100$$

Germination test and cotton seed borne mycoflora investigation

Germination test of seed samples were done on moistened blotting paper and in field condition**.** In the laboratory, 400 seeds were placed at the rate of 20 seeds per Petri plates with moistened blotters. Germination was recorded at 5, 7 and 14 days after sowing. Normal seedlings, abnormal seedling and dead seeds were counted separately and expressed in percentage. Also an experimental field was set up in September 2016 on a one-year crop history fallow in Daloa. It was subjected to the same cotton crop management technique as the one recommended by research with some slight modifications [2]. The seeds used were not treated before sowing. However, the replacement of empty hills was done by disinfecting the seeds with a fungicide-insecticide mixture (Thiram + Imidacloprid). Seedling were counted and included normal seedlings, abnormal seedlings, and dead seeds (empty hills).

The analysis of mycoflora associated with seed and abnormal seedlings resulting from the germination test was carried out with reference to the Incubation methods. This method consisted in incubating plant organs and seeds for five–seven days at 20–25°C in Petri dishes containing PDA media (Potato Dextrose Agar) under 12 h light-dark cycle. The proliferation of bacteria in cultures was inhibited by adding chloramphenicol to the media at a rate of 0.5 g/L [18]. After the incubation period, fungi developed on each seed are examined by mean of stereomicroscope for the determination of the morphological characters of conidia or spores. 12 h

DATA ANALYSIS

Collected data of both field and laboratory experiment were analyzed following the analysis of variance (ANOVA) using the R software ((The R Project for Statistical Computing). Afterwards, Kruskal Wallis, a non-parametric version of a one-way ANOVA, was used to compare levels of factor independently. As for fungal species, their identification was made using fungal systematics [19-21].

RESULTS

Particularity of collected seeds

The different samples collected from 10 producers were grouped per variety. In the end, 3 varieties (Y301AR3, Y764AR3, Y331BR4) were the ones grown over the 10 (table-1). They were all improved varieties resulting from research (Centre National de Recherche Agronomique). Generally they are supplied in the form of basic seeds to cotton companies. The seeds are then multiplied by the certified-grower of supervision services that recommend them and provide them free of charge to farmers. The seeds of Y301AR3 and Y764AR3 varieties were 3rd generation (R3) certified seeds while Y331BR4 belonged to the fourth generation.

Dry Inspection and moisture content of 3 varieties cotton seed samples

Results of three components such as pure seed, inert matter and moisture content of cotton seed are presented in Table 2. In the samples studied, a lack of other seeds was noted. The purity level of seed lots was well above 90%. The moisture content of the seeds was 10% and attested to the good physiological quality of the seeds. So, these three components were not differed significantly from seed samples of the cotton varieties.

Table-2: Purity test and moisture content (MC) of cotton seed collected from 10 Farmers field

Variety	Purity test (%)		MC (%)
	Pure seed	Inert matter	
Y301AR3	97	3	10,05 ±0,3
Y331BR4	96,27	3,73	10,24 ± 0,9
Y764AR3	97,01	2,99	10,07 ± 0,44
p-value	NS	NS	NS

Total seed tested = 100 g/sample, 3 samples were tested of each treatment

Germination test in both laboratory and field condition

Cotton seed germination test with respect to normal, abnormal seedling and dead seed significantly affected all the tested varieties (Tableau 3). The germination rate (normal + abnormal seedlings) in the laboratory condition of the 3 seed lots was < 40% at 14 days after sowing with a low rate of normal seedlings. The latter were 27.75% for the Y301AR3 variety, 16.5% for the Y764AR3 variety and 4% for the Y331BR4 variety. The dead seeds were the most numerous in the lots and were 95 % for the Y331BR4 variety, 79.50 % for the Y764AR3 variety and 61 % for the Y301AR3 variety. The abnormal seedlings were characterized by collar browning or even rot (figure-1).

On the experimental field, germination test consisted in observing the emergence rate after sowing. In that respect, 3 groups were distinguished (Table 3). These included hill groups left empty 14 days after sowing, normal seedling groups and abnormal seedling groups, that is, seedlings with collar browning or rot or non-vigorous seedlings that eventually collapse and die later on. Thus, the normal seedling rate was 12.75% for the Y301AR3 variety, 43.75% for the Y764AR3 variety and 21% for the Y331BR4 variety. Empty hills were found at a rate of 40.5% for the Y301AR3 variety, 27.75% for the Y331BR4 variety and 32% for the Y764AR3 variety. As for abnormal seedlings, their rates were 46.75% for the Y301AR3 variety, 51.25% for the Y331BR4 variety and 24.5% for the Y764AR3 variety. The statistical analysis of these data showed a significant difference between the 3 varieties in terms of normal seedling rate. It was very low for the Y301AR3 variety (12.75%). The germination rate was subsequently improved after seed disinfection with Thiram + Imidacloprid mixture. Thus, it rose to 71.25% for the Y301AR3 variety, 76.25% for the Y331BR4 variety and 74.25% for the Y764AR3 variety. The statistical analysis showed no significant differences between the germination rates of the varieties after seeds disinfection.

Table-3: Germination status of different varieties as affected by methods and treatment

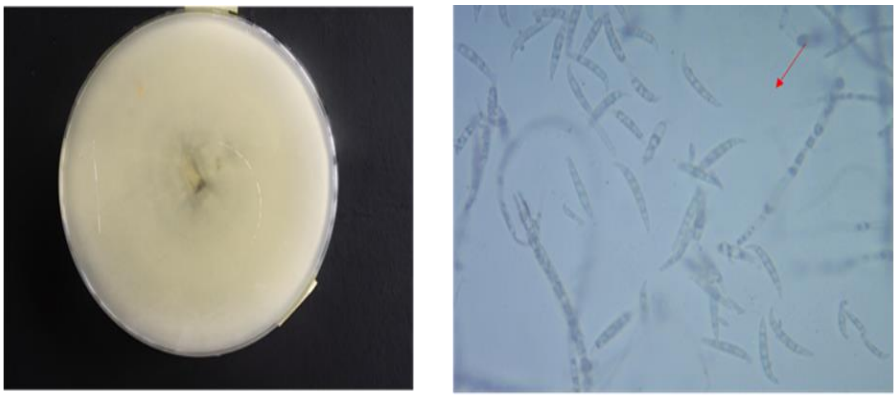
varieties	L				F				F1		
	N	**AB**	**D**		**N**	**AB**	**E**		**N**	**AB**	**E**
Y301AR3	$27,75^{b}$	$11,25^{b}$	61^{a}		$12,75^{a}$	46,75	40,5		71,25	4,5	24,25
Y331BR4	4^{a}	1^{a}	95^{b}		21^{b}	51,25	27,75		76,25	4,5	19,25
Y764AR3	$16,5^{b}$	4^{ab}	$79,50^{b}$		$43,50^{b}$	24,5	32		74,25	6,25	19,5
(P-value)	**$32.10^{-‘}$**	**87.10^{-5}**	**$1,22.10^{-5}$**		2.10^{-5}	NS	**NS**		NS	NS	NS

In a column, means followed by a common letter are not significantly different at the LSD 5%. L: laboratory condition; F: Field condition; F1: field condition after seed treatment; N: Normal seedling; Ab: Abnormal seedling; D: Dead seed. E: empty hill

Fig-1: Abnormal seedlings (rotting root) from cotton seed A & B: laboratory condition, C: Field condition

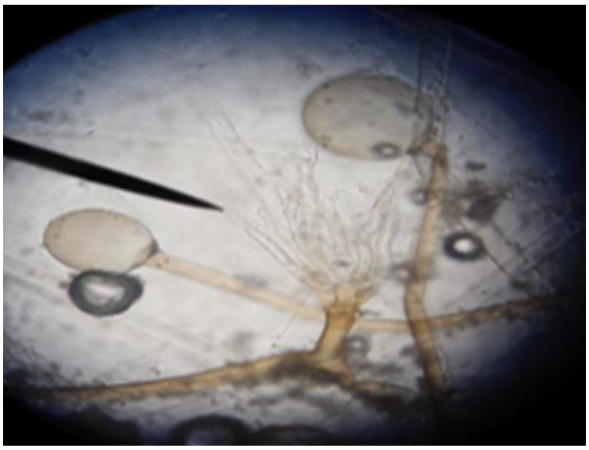
Health test of cotton seed samples (incubation method)

The assessment of the mycoflora of cotton seed and seedlings which showed an abnormality during the germination test has helped identify 6 fungal genera (figure2). The associated fungi were Fusarium sp, Aspergillus sp, Curvularia lunata, Rhizomucor sp, Macrophomina phaseolina, Paecilomyces sp. Among them, 3 were pathogenic. They included Fusarium oxysporum, Curvularia lunata, and Macrophomina phaseolina. No difference was observed between the varieties with respect to the species observed (data no showed).

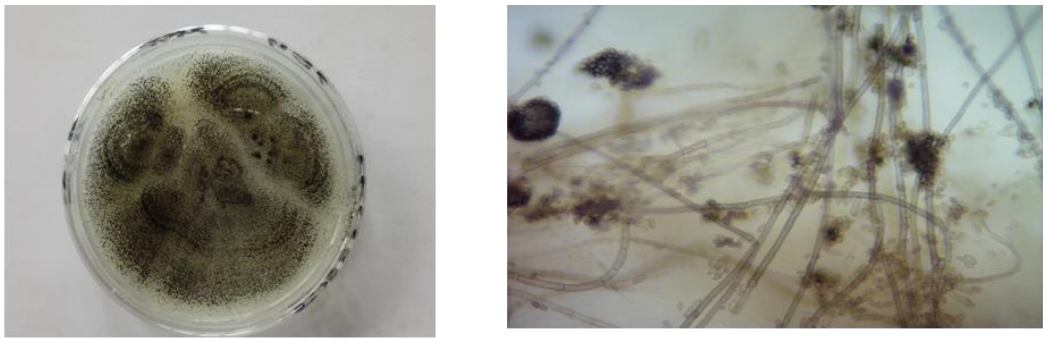


Fusarium sp.

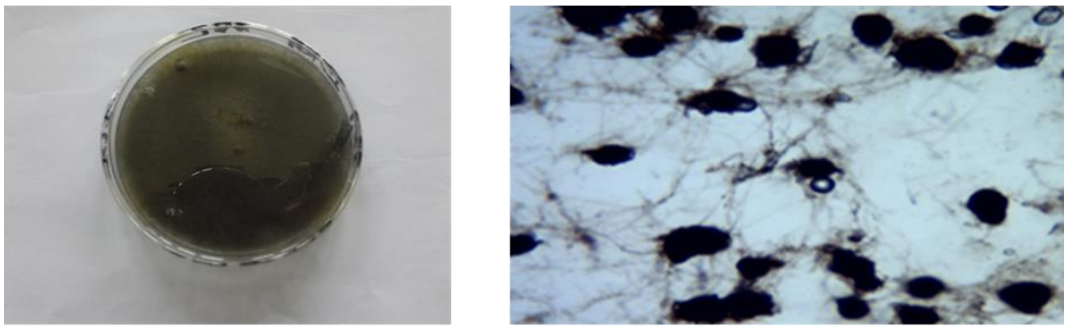
Curvularia lunata



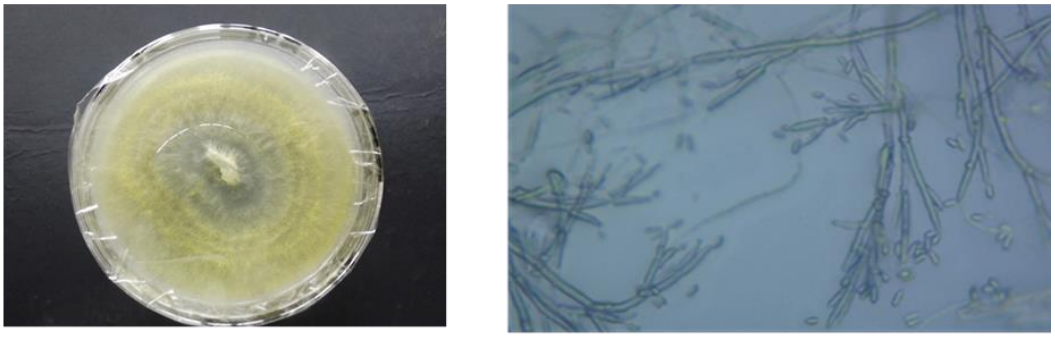
Rhizopus sp.



Aspergillus sp.



Macrophomina phaseolina



Paecilomyces sp.

Fig-2: Macroscopic and microscopic views of isolated 6 fungal genera associated to cotton seed

DISCUSSION

Surveys carried out in a dozen cotton fields during the crop year 2016 revealed that the supervision systems of the sector are omnipresent to support farmers. This crop is strongly recommended in the area by large organizations which provide seeds to farmers. The three varieties obtained from farmers were all improved varieties resulting from research but left at farmers'. The assessment of their health quality is a means of prevention and/or important fight against field crop diseases. Cotton seeds can in fact host the pathogens responsible for major epidemics, the appearance of which coincides with Crop development [12]. In case of severe attack by pathogens, the rate of infected seeds can reach 100% [22]. The use of good quality seeds is therefore a must to avoid any supply of external inoculum.

Assessment of seed species purity of Y301AR3, Y764AR3 and Y331BR4 cotton varieties showed clean lots free from weed and other crop seeds. The debris and the inert matter found there were in low quantity, meeting thus the ISTA [25] standards. Cotton seed multipliers have therefore made every effort to obtain maximum varietal purity. According to authors, the need for the use seeds free from foreign species appears as one of the crop success factors [23]. However, moisture content is the most important factor determining the rate at which seeds deteriorate [24]. The moisture content of the seeds studied was 10%. This rate, however interesting, is not ideal for long-term storage of oilseeds such as cotton. Indeed, the ISTA recommends for their storage in an active collection that their content be between 3 and 8% so as to maintain a good health condition. For this purpose, the results of the seed germination test of the 3 varieties in the laboratory as well as on-farm showed a normal seedling rate well below 30%. These results could be explained by a contamination of the seeds by both saprophytic (Aspergillus sp., Rhizomucor sp, Paecilomyces sp) and

pathogenic (Fusarium sp., Curvularia sp., Macrophomina sp) fungi.

Seeds contaminated with saprophytic fungi do not germinate or would produce weak or abnormal seedlings. Indeed, saprophytic fungi can cause either seed sterility or reduce the energy of germination or cause the formation of abnormal chains that can cause the death of seedlings. Their presence on cotton seeds could also explain the high rate of rotten seeds found in the laboratory. As for pathogenic fungi such as Fusarium sp, Macrophomina sp, Curvularia sp, they occur in culture and infect the seeds in the field before harvest. Seed moisture greater than 15% contribute to their development. These fungi are involved in emergence gaps and seedling damping-off generally observed in crops. They can be associated with the high rate of abnormal seedlings observed in the laboratory and in experimental plots. The use of seeds infected with these parasites can therefore reduce the potential yield of cotton crops. The poor health quality of cotton seeds justifies that they must be treated before sowing. This was confirmed on the experimental plot where the first sowing with non-disinfected seeds resulted in poor emergence and many empty hills. Thereafter, the disinfection of seeds with Thiram + Imidacloprid mixture as recommended made it possible to protect them against any aggression and to obtain a good emergence. The use of selected disease-free seeds is therefore essential for successful cotton cultivation. Quality seeds are one of the most important inputs in the cotton production chain.

CONCLUSION

This work is a contribution to the knowledge of the health condition of the seeds used in cotton cultivation in Côte d'Ivoire. It has helped show that the cotton sector's supervising systems are present in West Central where the farmers benefit from additional supervision from sowing to harvesting. However, although the seeds used by farmers in West Central Region are certified seeds, the analysis of their health condition must be taken into account in certification programs. Indeed seed analysis has shown 6 fungal genera which alter their germination and therefore could reduce the potential yield of cotton crops. The introduction of seed health testing in the laboratory is thus strongly recommended in order to improve seed quality and, consequently, to increase crop yields by preventing the spread of diseases. This work must imperatively extend to seeds grown throughout the cotton basin so as to meet the challenge of optimal cotton yield in Côte d'Ivoire.

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