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Seed-borne fungi of some soybean varieties from Cameroon and impact on seed germination and seedling vigour index

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Abstract

An investigation to detect the seed-borne fungi of soybean varieties cultivated in Cameroon and their effect on seed germination and seedling vigour was conducted in the Phytopathology Laboratory of Plant Protection Department, University of Dschang from April to July 2016. Seven varieties/line were collected, five from IRAD station of Foumbot and two from FAR of Faculty of Agronomy and Agricultural Sciences. Blotter method and agar plate were used to detect associated fungi of soybean seed and to evaluate the percentage of germination. Altogether, fourteen fungi comprising nine genera namely *Aspergillus flavus, A. niger, Botritis* sp, *Cladosporium*sp, *cercospora* spp, *colletotricum*sp, *Fusarium moniliforme, F. solani, F. oxysporium, penicellium* sp, *Phomopsis* sp, *Rhizopus stolonifer. Chaetomium* sp *and Mélanospora zamiea,* were detected from soybean seed samples. The germination of seed samples varied from 53.5 (TGX-1910-14E) to 98.9 % (TGX-1835-10E and ENGOPA 314). Germination of seeds was directly related to the prevalence of fungi associated with the seed. The vigour index varied from 818 for ENGOPA 316 to 1456 for HOULA 1.ENGOPA 316 and TGX-1835-10E gave the least vigour index significantly different with all of other varieties. Important pathogenic fungi recorded which are responsible of lowering of germination percentage of some soybean varieties, need more control for enhancing soybean productivity.

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Introduction

Soybean, (*Glycine max* (L) Merril) is a world wide economic crop and the most important cultivated legume with hundreds of food, feed and industrial usages. The crop can be grown in tropical, sub-tropical as well as the temperate regions for food and economic needs (Halima, 2000; Sneller, 2003) ; and has been introduced in Cameroonsince. It is a primary source of vegetable oil, protein concentrates and starch (Anon, 1994; Anonyme, 2013 ; Venugopal *et al.*, 2015). Soybean is an excellent source of major nutrients, about 40% of dry matter is protein and 20% fat (CaldWell 1973).

Due to these nutritional qualities, it is highly used in human and animals nutrition. In Cameroon, production is mainly carried out in the north and west regions despite the great potential of the countries. Soybean cultivation in the north region was encouraged by IRAD and SODECOTON in 2007 due to the decrease in cotton bean production in the northern regions of the country.

National production of Cameroonin 2010 was12544tons of soybean with an average productivity of 1.28 ton/ha (AGRISTAT, 2012), wide below the global average which is 2.49 tons/ha during the year 2010.

Among several other factors contributing toward slow yield of this crop in Cameroon, the most important are diseases. Most of the diseases of soybean are carried though seed and cause enormous losses to the crop. Microorganism splay an important role in affecting the quality of seed, of which fungi are the largest group. Seed-borne diseases caused by fungi are relatively difficult to control as the fungal hyphae get established and become dormant (Butt *et al.* 2011).

Soybean seeds are thus infected with various seed borne microorganisms, including fungi, bacteria and viruses. Many researchers have reported that *Aspergillus flavus, A. niger, Cercospora kikuchi, Macrophomina phaseolina, Fusarium oxysporum, F. solani,* and *F. moniliforme, Phomopsis* sp*, Cladosporium* sp*, Rhizopus* sp, *Curvularia* sp, *Alternaria* sp, *Colletotrichum dematium, Phoma* spp etc. are the most associated soybean fungi (Nasreen, 2003; Krishnamurthy *et al.,* 2006 and 2008; Impullitti *et al.,* 2013; Lakshmeesha *et al.,* 2013 and 2014; Venugopal *et al.*, 2015). These fungi are responsible for the loss of germination potential, loss of seedling and rot of seed. Disease free quality seed production in soybean is of utmost importance to sustain productivity and maintain the quality of the crop. Unfortunately, information on seed-borne fungi associated with soybean seeds is not more documented in Cameroon. The present investigation has therefore been undertaken to detect the seed-borne fungi associated with soybean seeds of some recently introduced and local varieties.

Materials and methods

The experiments on most of the seed borne fungi associate with soybean and their effects on seeds germination was conducted in the phytopathology laboratory of the department of Plant Pathology, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon, during April 2016 to July 2016.

Seed samples collection

Seven varieties of soybean have been collected. Five from IRAD station of Foumbot, two from FAR (*Fermed'Applicationet de Recherches*) of Faculty of Agronomy and Agricultural Sciences of University of Dschang (MAGBA and TGX-1835-10E). Such varieties have been also grouped in three: local varieties (HOULA 1, MAGBA), introduced varieties (ENGOPA 301, ENGOPA 314, ENGOPA 316; all these are from Brazil), and selected varieties/line by IITA and IRAD (TGX-1910-14F, TGX-1835-10E).

Detection and identification of fungi

Seeds samples took randomly from the seven varieties have been analysed for detection of seed-borne fungi by blotter paper and agar plate methods following International Rule for Seeds Health Testing (ISTA, 1996) with some modifications. 200 seeds from each

sample were surface disinfected by soaking in 2% of sodium hypochlorite solution for three minutes and rinsed with sterile distilled water for five minutes. The seeds were placed in Petri dishes(10 seeds per Petri dish). The Petri dishes with seeds were then incubated at 22°C ± 2 for 7 days with 12 hours of photoperiod in a room. After incubation of the seeds, germination and fungi associated to the seeds have been recorded. Each of the incubated seeds was examined under steriobinoculer microscope to ascertain the presence of fungi and morphological characterization. A slide was prepared for each colony that could not be identified base in their morphological character for proper observation with compound microscope. Identification was confirm by using a standard key as described by Rubert and Streets (1982).

Seed germination

The number of germinate seed were counted after 7 days of incubation in a room. Percent seed germination was then expressed as number of seeds germinated over total number of seeds planted.

Seedling vigour index

Twenty seeds per variety were collected randomly and sown in germination plate. Then the seedling length was measured after ten days. Seedlings vigour index was calculated using the formula as method of Venuturla Bharathi (2013).Seedling vigour index = Mean seedling length (cm) × germination percentage (%).

Statistical analysis

The data collected on different parameters were subjected to Analysis of variance (ANOVA) using SPSS software version 17 and the mean for all treatments were separated using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$.

Results and discussion

Prevalence of seed-borne fungi of soybean

The level of infection of seeds is relatively high for all varieties. Thirteen species of fungi belonging to ten genera were isolated from soybean seeds using both agar plate and blotter paper method.

Table 1. General seeds heath and their germination percentage.

Varieties	%Germination	%Infection	% Co-infection	Frequencies RS	Fr IRS
ENGOPA 314	98.9	67.4	17.2	9.5	66.7
ENGOPA 316	97.5	42.0	1.2	37.5	48.0
ENGOPA 301	63.5	79.0	16.5	32.0	29.7
HOULA 1	71.1	70.5	22.4	28.4	27.8
TGX-1910-14F	53.5	84.9	15.3	10.8	40.0
MAGBA	97.5	42.0	17.2	2.0	100.0
TGX-1835-10E	98.9	67.4	1.2	1.6	33.3

* Where RS refers to rotten seeds, IRS refers to infected rotten seeds.

The isolated fungi were *Aspergillus flavus, A. niger, Botritis* sp, *Cladosporium* sp,*Cercospora*sp, *colletotricum* sp*, Fusarium moniliforme, F. solani, F. oxysporium, Penicellium* sp*, phomopsis sp, Rhizopus stolonifer. And Mélanospora zamiea.* Most of these fungi have been reported early by Moss and Smith (2006);Shovan *et al.* (2008); Ramesh *et al.* (2013); Ibrahim (2015);Venugopal *et al.*(2015).

With respect to the total mean of seed infection, the line selected by IITA and IRAD (TGX-1910-14F) showed high infection rate of 84.9%among all the varieties; it is also the most infected breeding line among the two considered in this study (table 1). About local varieties, HOULA 1 showed the greatest infection percent with 70.5% of infection. Concerning introduced varieties, ENGOPA 301 showed the greater infection rate (79%). Seeds collected from the FAR of University of Dschang showed the least percentage of infection ranged from 42% to 67.4% for

MAGBA and TGX-1835-10E respectively, whereas seeds collected from Foumbot were most infected with percentage of infection ranged from 42%, 67.4%, 79%, 70.5%and 84.9% respectively for ENGOPA 316, ENGOPA314, ENGOPA 301, HOULA 1 and TGX-1910-14F.Globally TGX-1910-14F was the variety which has the highest rate of infection (84.9%), while the least rate of infection has been recorded on MAGBA and ENGOPA 316 (42% for each one).

Table 2. Prevalence (%) of seed born fungi associated to soybean seeds.

Fungal species	Blotter method						PDA							
	MAGBA	TGX-1835	ENGOPA 316	ENGOPA 314	HOULA 1	TGX-1910	ENGOPA 301	ENGOPA 316	ENGOPA 314	ENGOPA 301	HOULA 1	MAGBA	TGX-1835	TGX-1910
Cercospora sp	-	1.1	41.4	25.0	18.5	33.3	20.0	50.0	1.0	2.0	42.1	42.9	30.0	15.0
Fusarium spp	2.9	1.1	41.4	17.1	13.0	22.2	20.0	-	-	-	-	-	-	-
A. flavus	8.8	25.6	1.0	7.9	11.1	5.6	6.7	-	7.0	1.0	5.3	33.3	30.0	5.0
Phomopsis sp	5.9	-	10.1	3.9	5.6	7.4	6.7	-	-	-	-	-	3.3	-
Rhizopus stolonifer	-	4.4	-	11.8	14.8	-	-	-	-	5.0	-	-	-	-
A. niger	26.5	42.2	-	7.9	3.7	1.9	4.4	5.6	5.0	6.0	-	4.8	3.3	-
Cladosporium sp	52.9	25.6	6.1	17.1	16.7	7.4	17.8	16.7	5.0	1.0	15.8	19.0	13.3	20.0
Collectotricum lin	-	-	-	-	7.0	-	20.0	-	-	-	-	-	-	-
Botritis	-	-	-	9.2	3.7	20.4	4.4	-	4.0	-	-	-	-	-
Melanospora zamiae	2.9	-	-	-	-	1.9	-	-	-	-	-	-	-	-
Penicillium sp	-	-	-	-	-	-	-	27.8	15.0	8.0	36.8	-	20.0	60.0
Chaetomium sp	-	6.0	4.5	-	6.0	5.0	-	-	-	-	-	-	-	-

The rate of co-infection of seeds is high in HOULA 1 (22.4%), and the highest rate of infected rot seeds has been record on MAGBA with 100% (all rotten seeds are infected) (table 1).

The differences in occurrence of seed mycoflora in soybeanseedsamplescollectedfromdifferentlocalitiesm aybeattributed to the variations in moisture content of the seed and storage conditions (Temperature, Relative humidity and Light) adopted by the farmers. Mycoflora of seed varied from place toplace due to change in conditions prevailing during seed development, harvesting and storage (Dwivedi and Ram, 2014; Venugopal *et al.,* 2015).

Table 3. Prevalence of fungi by seed samples origin.

Fungalspecies	Blotter method			PDA		
	Local Var	IITA/IRAD	Introduced	Local Var	IITA/IRAD	Introduced
Cercospora sp	9.26	17.22	28.80	42.48	22.50	17.67
Fusarium spp	7.95	11.67	26.17	-	-	-
A. flavus	9.97	15.56	5.19	19.30	17.50	2.67
Phomopsis sp	5.72	3.70	6.91	-	1.67	-
Rhizopus stolonifer	7.41	2.22	3.95	-	-	1.67
A. niger	15.09	22.04	4.11	2.38	1.67	5.52
Cladosporium sp	34.80	16.48	13.65	17.42	16.67	7.56
Collectotricum lin	3.50	-	6.67	-	-	-
Botritis	1.85	10.19	4.55	-	-	1.33
Melanospora zamiae	1.47	0.93	-	-	-	-
Penicillium sp	-	-	-	18.42	40.00	16.93
Chaetomium sp	3.00	5.50	1.50	-	-	-

Among isolated fungi (table2), *Cercospora* sp and *Fusarium* sp were most prevalent fungi in all the seed samples from Foumbot with 41.4% and 41.4% respectively for ENGOPA 316, the least prevalent was *A. flavus*. Similarly for TGX-1910-14F variety, *Cercospora* sp, *Fusarium* sp and *Botritis* sp were most prevalent with respectively 33.3%, 22.2%, and 20.4%, when *Melanospora sp* and *A. niger* were least prevailing with 1.9% for each one. For ENGOPA 301, *Cercospora* sp (20*%), Fusarium* sp(20%),

Colletotrichum sp (20%) and *Cladosporium* sp (17.8%)were most prevalent.InTGX-1835-10E variety from the FAR, *A. niger* (42.2%) *A. flavus* (25.6%) and *Cladosporium* sp(25.6%) were most important. For the variety MAGBA (from the FAR) *Cladosporium* sp (52.9%) and *A. niger* (26.5%) are the prevailing fungi and the least prevailing fungi were *Melanospora* sp and *Fusarium* sp with 2.9% for each one. *Cladosporium* sp was most prevalent fungus in local varieties when *A. niger* was predominant in line of IITA/IRAD, and *Cercospora* sp is dominant in introduced varieties (**Error! Reference source not found.**). Blotter method has shown a large number of fungi than PDA (**Error! Reference source not found.**2).Similar observation has been mentioned early by Venugopal *et al.* (2015).

Table 4. Vigour index values of the varieties.

Varieties	Vigour index	Percentage of germination	Seedling length
TGX-1835-10E	848.1^{a}	98.9^{a}	11.7^{a}
MAGBA	1241^{b}	97.5^{b}	13.8^{ab}
ENGOPA 316	818.6^{a}	97.5^{b}	13.4^{ab}
HOULA 1	1456^{b}	71.1^{c}	18.5^{c}
TGX-1910-14F	1336^{b}	53.5^{d}	15.8^{bc}
ENGOPA 314	1248^{b}	98.9^{a}	15.6^{bc}
ENGOPA 301	1166.5^{b}	63.5^{e}	16^{bc}

Means in a column for each variety followed by the same letters are not significantly different according to Duncan New Multiple range Test (P = 0.05).

Seed germination percentage and seedling vigour index

The percentage of germination and seed vigour index are presented in **Error! Reference source not found.**. The varieties/line selected by IITA/IRAD, TGX-1835-10E and introduced variety ENGOPA 314 have shown high percentage of germination up to 98.9% for each one. Varieties TGX-1910-14F and ENGOPA 301 have the least germination percentage respectively 53.5% and 63.5%. This result can be justified by the fact that these two varieties showed the highest fungal infection of seeds as shown in Table 1. General seeds heath and their germination percentage and the presence of some pathogenic fungi like *Fusarium* sp, *Colletotrichum* sp, *Cercospora* sp, *Phomopsis* sp. were noted. Some authors reported that the presence of these fungi can reduce seed germination (Shovan *et al.,* 2008; Fagbohun and Lawal, 2011; Ibrahim, 2015).The highest seedling vigour index have been recorded on varieties HOULA 1 followed by TGX-1910-14F and ENGOPA 314 with respectively 1610, 1336 and 1248 as shown in **Error! Reference source not found.**. The least values have been recorded on varieties TGX-1835-10E and ENGOPA 316 with respectively 848 and 818. It is known that HOULA 1 is local variety cultivated in arid and semi-arid zone.

The mycoflora associated with soybean seed samples were found to reduced seed quality parameters. Significant differences in mean seed germination, mean seed vigour index was recorded. Seed borne fungi present in soybean produced seed rots, seedling blights and decreased quality and quantity of soybean besides causing germination failures. Mehrotra and Aggarwal (2003) reported that such fungi could seriously retard seed germination through softening and necrosis of tissues. They also confirmed the association of seed-borne fungi with seed viability, wilting of plants and stem flaccidity. Incidences of several pathogenic seed-borne fungi on seeds have been reported by Leslie *et al.* (2005) and Anjorin *et al.* (2008). The factors influencing the development of seed-borne fungi include the moisture content of

the seed, prevailing temperature, storage period and degree of seed invasion with the pathogen). Others are level of host genetic resistance, activities of insects and mites and amount of foreign materials in the seed lot (Miller and Trenholen, 1994).

Conclusion

Finally, the results of the present study reveals that many fungi are associated to soybean varieties cultivated in Cameroon. Mycoflora and prevalence of some fungi varies with the origin of seeds as well as the conditions prevailing during seed development harvesting and storage. Since Breeding line and introduce have the great rate of infection, some of them also have the best percentage germination of seed. Important pathogenic fungi recorded which are responsible of lowering of germination percentage of these varieties, need more control for enhancing soybean productivity.

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