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Seed-borne Fungi Detected in Sugar Beet Seeds Imported into India during Last Three Decades

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Abstract

Quarantine processing of 1,111 samples of germplasm and inbred lines of sugar beet seed imported into India from 1976 to 2005 resulted in the interception of 10 pathogenic fungi including *Uromyces beticola*, the beet rust pathogen which is not yet reported from India. Other seed-borne fungi of high economic significance that were detected included *Colletotrichum dematium*, *Fusarium solani*, *Pleospora betae*, and *Verticillium albo-atrum* as well as other fungal pathogens for which sugar beet is a non-host crop, such as *Drechslera halodes*, *D. rostrata*, *D. sorokiniana*, *Cephalosporium* sp., and *Pestalotia* sp. Various fungi detected in sugar beet seed imported from different countries are presented and their economic significance is discussed. Seeds contaminated with *U. beticola* were disinfected by dipping them in concentrated sulphuric acid for 1 min while those infected with *P. betae* were disinfected by hot water treatment at 50°C for 30 min.

Introduction

Sugar beet (*Beta vulgaris* L.) is second only to sugarcane as the major source of the world's sugar. Its culture is a comparatively recent development, whereas sugarcane culture dates from antiquity. The first beet-sugar factory was built in Silesia (now in Poland) in 1802. In the last century, sugar beet accounted for about two-fifths of the world's sugar production and the major producers were the former Czechoslovakia, France, Germany, Italy, Poland, Romania, Turkey, the UK, the USA, and the former USSR.

The Plant Quarantine Division at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi is the nodal agency for quarantine clearance of imported germplasm intended for research purposes. A large number of economically important fungal pathogens have been intercepted in these imported germplasm including several that are not reported from India (2). Sugar beet seeds in the form of germplasm and inbred lines are being introduced into India for developing cultivars suitable for local agro-climatic conditions. There is always a risk of inadvertently introducing exotic pests associated with such imports. Twenty-two pests (14 fungi, 2 bacteria, 5 viruses, and 1 nematode) are listed as associated with sugar beet seeds (13). Blackleg caused by *Pleospora betae* (Berl.) Nevodovsky, leaf spot caused by *Cercospora beticola* Sacc., and beet rust caused by *Uromyces beticola* (Belynnck) Boerema et al. are the important fungal diseases of beet from a quarantine point of view as they are highly seed-transmitted. *U. beticola* was introduced into Canada through seeds imported from Europe (8). In this paper, pathogenic fungi intercepted in sugar beet seeds introduced from 1976 to 2005 are reported and their economic significance is discussed. An analysis of data on the level of infection of various fungi in imported sugar beet seed is made by dividing the 30 years of results into three decades. The Decade I is from 1976 (the year of inception of NBPGR) to 1985. The Decade II is from 1986 to 1995 and the

Decade III is from 1996 to 2005. This categorization is done in order to analyze the increase or decrease in the risk associated with such imports over the years.

Processing of Germplasm for Quarantine Clearance

During the last three decades a total of 1,111 samples of germplasm and inbred lines of sugar beet seed from 21 countries comprised of 150 consignments were imported into India for developing cultivars/hybrids suitable for local agro-climatic conditions. All the imported samples of sugar beet seeds were subjected to the washing test for the detection of *U. beticola*, beet rust (1), and subsequently to the standard blotter test prescribed by ISTA for the detection of other seed-borne fungi as described below. The number of samples imported from various countries and the fungi detected are presented in Table 1.

Table 1. Seed-borne fungi detected in sugar beet introduced into India during 1976-2005^x.

Source/ country	Samples received	Fungi intercepted ^y				
		<i>C. dematium</i>	<i>F. solani</i>	<i>P. betae</i>	<i>U. beticola</i>	<i>V. albo- atrum</i>
Belgium	13	1 (8) ^z		5 (39)		2 (15)
Denmark	127		1 (1)	3 (2)	4 (3)	
Germany	198		1 (1)	7 (4)	6 (3)	
Hungary	24		3 (13)	7 (29)		3 (13)
Iran	32		2 (6)	3 (9)		1 (3)
Italy	112	2 (2)	2 (2)	15 (13)	5 (5)	
Japan	4			4 (100)		
The Netherlands	51				6 (12)	
Poland	40		5 (13)	22 (55)		8 (20)
Romania-	5	4 (80)		4 (80)		5 (100)
Russia	1			1 (100)		
Sweden	67		2 (3)	23 (34)	2 (3)	6 (9)
The UK	34			1 (3)	3 (9)	
The USA	211	1 (1)	3 (1)	4 (2)	7 (3)	
The USSR (former)	101		1 (1)	5 (5)	11 (11)	1 (1)

^x Of the 1,111 samples introduced, 89 samples received from 7 other countries did not reveal any fungi.

^y Other fungi intercepted were: *Cephalosporium* sp. (Italy); *Drechslera halodes* (Drechsler) Subram. & Jain (Italy); *Drechslera rostrata* (Drechsler) Richardson & Fraser (Italy); *Drechslera sorokiniana* (Sacc.) Subram & Jain (Belgium, Poland); and *Pestalotia* sp. (Italy).

^z Figures outside the parentheses indicate the total number of samples found infected, and within parentheses, the percent of samples found infected.

Washing test. Four hundred seeds from each sample of sugar beet were picked and placed in 16 test tubes each containing 20 ml of distilled water (25 seeds in each tube). The contents of each tube were stirred for 2 min on a vortex stirrer. The liquid was decanted in plastic Petri plates and observed for *U. beticola* spores under stereo-binocular microscope and initial observations were recorded (1). These seed-washings were centrifuged in tubes at 5000 rpm for 10 min. The supernatant was discarded and three drops of distilled water were added to each pellet. Slides were prepared from the contents of each tube and examined under the compound microscope for the presence of rust spores and final observations of washing test were recorded (1).

Blotter test. Four hundred seeds from each sample of sugar beet were placed on three layers of moist blotters in 16 plastic Petri plates (25 seeds in each plate, 1 in the centre, 9 in the inner circle, and 15 in the outer circle). These plates were then incubated for 7 days at $20 \pm 1^\circ\text{C}$ under alternating cycles of 12-h light (by Philips 40-W fluorescent day light tubes) and 12-h darkness. Fungi associated with seeds were identified and recorded on the eighth day under stereo-binocular microscope. When the fungal growth could not be identified under stereo-binocular microscope, slides were prepared and observed under compound microscope (2). The blotter test is being routinely used for the detection of seed-borne fungi in more than 60,000 samples of germplasm and trial material that is introduced every year by NBPGR. Sample size for sugar beet germplasm in the blotter test was usually 400 seeds as we received enough quantity of seed in this crop. In other crops, sample size was dependent on the quantity of seed received (2).

The choice of disinfection method used depended on whether the pathogen was present on the seed surface and/or along with the seed, as in the case of *U. beticola*, or present inside the seed as well, as in the case of *P. betae*. Samples of sugar beet seeds contaminated with *U. beticola* were disinfected by dipping the seeds in concentrated sulphuric acid (98%) for 1 min followed by a wash under running tap water as described earlier from this laboratory (12). This technique completely eliminated this rust pathogen from contaminated beet seed (12). Seeds infected with *P. betae* were disinfected by hot water treatment (HWT) at 50°C for 30 min and subsequently sun dried. Seed samples found contaminated with *U. beticola* as well as infected with *P. betae* were disinfected by concentrated sulphuric acid treatment followed by HWT (12). Effectiveness of acid treatment and HWT was tested by washing test (1) and blotter test (12), respectively. Samples found infected with other seed-borne fungi were given fungicidal seed dressing with thiram 2 g a.i./kg seed+ carbendazim 500 mg a.i./kg seed so as to minimize the chances of spread of fungi along with imported germplasm.

The washing test revealed (Table 1) the presence of urediospores and teliospores of rust, which had characteristics resembling those of the spores of *U. beticola* (11). *U. beticola* was first intercepted in India during 1983 in seeds of sugar beet germplasm imported from the USA and Italy. Data presented in Table 1 indicates that this rust was intercepted in 44 samples. These samples were imported from Denmark (four), Germany (six), Italy (five), the Netherlands (six), Sweden (two), the UK (three), the USA (seven), and the former USSR (eleven). Further, a decade-wise analysis of data showed that *U. beticola* presence remained unchanged in samples from the UK and the USA in Decades I and II, however, in Decade III in samples from the USA its presence was reduced by 80%. Likewise in samples from Germany, the infection levels reduced by more than half in Decade III as compared to the previous decade. Besides sugar beet seeds, *U. beticola* is reported worldwide on spinach beet [*Beta vulgaris cicla* (L.) Koch], wild beet (*Beta procumbens* C. Sm.), and mangold [*Beta vulgaris vulgaris* (L.) L.] (11). However, it is not yet reported on any host in India. The chances of establishment of a pest upon introduction into the country increases when a greater number of hosts is reported. Severe forms of this rust have been reported from New Zealand, Canada, Denmark, Germany, and Israel (11). *U. beticola* is a macrocyclic, autoecious rust. Development of this disease is favoured by warm summer weather with frequent light rain or overnight dew because the urediospores can only germinate and infect leaves when surface moisture is present (3). Teliospores of the fungus overwinter on seed and serve as the primary source of inoculum for the crop in the subsequent year (10). Therefore, teliospore contaminated seed clusters must be considered as a potential risk for the introduction of the fungus into new areas since these spores retained viability on seed for 2 years (5). Beet rust was introduced into Canada from Europe through infected seeds (8). In view of the absence of beet rust in India and its seed-borne nature, the interception of *U. beticola* in sugar beet seed is of immense quarantine significance. Rust control by chemicals could give a yield benefit of 7% (3). Rust infection reduces the root yield and increases the levels of sodium and amino-nitrogen in the extracted juice (4).

The blotter test revealed a total of 9 pathogenic fungi (Table 1) including those of high quarantine significance and listed as seed-borne in sugar beet (13). *Colletotrichum dematium* (Pers.) Grove, the anthracnose fungus, was intercepted in 8 samples. These samples were introduced from Belgium (one), Italy (two), Romania (four), and the USA (one). Earlier this fungus was intercepted on introduced germplasm of several crops from Australia, Bangladesh, Belgium, Ethiopia, Hungary, Indonesia, Italy, Nepal, Nigeria, Philippines, Poland, Taiwan, Thailand, the UK, the USA, and Zambia (6).

Fusarium solani (Martius) Sacc. [= *Nectria haematococca* (Wollenw.) Gerlach] can cause several diseases including blight, wilt, and root or collar rot on various crops. It was intercepted in 20 samples of sugar beet seed. These samples were introduced from Denmark (one), Germany (one), Hungary (three), Iran (two), Italy (two), Poland (five), Sweden (two), the USA (three), and the former USSR (one). Further an analysis of data showed that *F. solani* infection in samples from Sweden was reduced by 30% in Decade II as compared to the earlier decade. Earlier, this fungus was intercepted on several crops from various countries (6). *F. solani* causes substantial economic damage as this fungus has a very wide host range.

P. betae, the blackleg fungus, was detected in 104 samples of sugar beet seeds received from Belgium (five), Denmark (three), Germany (seven), Hungary (seven), Iran (three), Italy (fifteen), Japan (four), Poland (twenty two), Romania (four), Russia (one), Sweden (twenty three), the UK (one), the USA (four), and the former USSR (five). The level of infection remained the same in Decade II and III in samples from Iran, but was reduced almost to half in samples from the USA in Decade II as compared to the previous decade. Likewise, it was reduced drastically in samples from Germany in Decades II (6.6% seed infection) and III (7.5% seed infection) in comparison to Decade I (100% seed infection). Infection level in samples from Sweden also reduced considerably in Decade II (14.2% seed infection) as compared to the previous decade (61.2% seed infection). This fungus is one of the primary causes of storage rot of sugar beet. Losses caused by *P. betae* in storage have been estimated as high as almost 8 million kg of sugar in some areas in some years, but losses vary (15). Chances of introduction of *P. betae* into new areas along with seed increases when seed infection levels are as high as 100% (9). Infection levels of *P. betae* in seeds was reduced from 27.5% to 4.5% after storing the seed at 10°C for 13 years at a relative humidity of 59% (7). This indicates that storage of sugar beet seed reduces chances of introduction of *P. betae* into new areas.

Verticillium albo-atrum Reinke & Berthold, the causal agent of Verticillium wilt in several crops was intercepted in 26 samples of sugar beet. These samples were introduced from Belgium (two), Hungary (three), Iran (one), Poland (eight), Romania (five), Sweden (six), and the USSR (one). Earlier, this fungus was intercepted in germplasm samples of several crops imported from many countries (6).

The blotter test also revealed pathogenic fungi for which sugar beet is a non-host crop, including *Cephalosporium* sp. in two samples of sugar beet seed from Italy, *Drechslera halodes* (Drechsler) Subram. & Jain in one sample from Italy, *D. rostrata* (Drechsler) Richardson & Fraser in one sample from Italy, *D. sorokiniana* (Sacc.) Subram. & Jain [= *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur] in one sample each from Belgium and Poland, and *Pestalotia* sp. in one sample from Italy. In the past, *D. sorokiniana* was intercepted on several crops from various countries (6).

Conclusion

The seed-health testing of 1,111 samples of sugar beet seed introduced during last three decades resulted in the interception of 10 pathogenic fungi of high economic significance including *U. beticola*, the beet rust pathogen which is hitherto not reported from India, and *P. betae*, the blackleg fungus, which causes great loss in storage. It is therefore concluded that for quarantine clearance of sugar beet seed, the washing test for the detection of *U. beticola* and the blotter test for other seed-borne fungi are important in order to avoid their introduction into hitherto uninfected areas or areas having limited distribution of these pathogens in the germplasm importing country. Seeds contaminated

with *U. beticola* were disinfected by dipping them in concentrated sulphuric acid, those infected with *P. betae* were disinfected by hot water treatment, while those found contaminated with *U. beticola* as well as infected with *P. betae* were disinfected by concentrated sulphuric acid treatment followed by HWT. Samples found infected with other seed-borne fungi were given fungicidal seed dressing with thiram 2 g a.i./kg seed+carbendazim 500 mg a.i./kg seed so as to minimize the chances of spreading fungi along with imported germplasm.

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