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Pathogenic fungi intercepted in introduced transgenics during 2006-2013

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Abstract

National Bureau of Plant Genetic Resources (NBPGR) New Delhi is the nodal agency for introduction of exotic germplasm including transgenics for research purpose in India. A range of transgenic crops have been developed across the world which are either herbicide-tolerant or insect resistant, or tolerant to drought/ stress or offering nutritional benefits, etc. India is also undertaking research in developing transgenics in different crops. During 2006-2013, a total of 4557 transgenic seed samples of various crops were introduced from different countries. Seed health testing of these samples for quarantine clearance resulted in the detection of eight pathogenic fungi viz., *Alternaria padwickii* in *Oryza sativa*; *Bipolaris maydis* in *Zea mays*; *B. oryzae* and *B. sorokiniana* in *O. sativa*; *Fusarium verticillioides* in *Arabidopsis thaliana*, *Brassica* spp., *Gossypium hirsutum*, *Solanum esculentum*, *O. sativa* and *Z. mays*; *F. oxysporum* f. sp. *vasinfectum* in *G. hirsutum*; *Phoma sorghina* in *O. sativa* and *Arabidopsis thaliana*; *Rhizoctonia solani* in *O. sativa* and *Zea mays*. All the 740 samples found infected were salvaged before their release to the indentors.

Key words – biosecurity–exotic pathogenic fungi – quarantine – seed health

Introduction

Transgenic crop plants have been developed by manipulating genes from diverse and exotic sources to confer resistance to insect pests and diseases, tolerance to herbicides, drought, improved post-harvest and food quality (with altered carbohydrate, protein, etc.) may play a pivotal role for developing countries with respect to food and nutritional security. In India, National Bureau of Plant Genetic Resources, New Delhi, is the nodal agency for quarantine processing of introduced germplasm including transgenic material for research purposes. Introduction of transgenics is of immense importance for research in crop improvement programmes, however, such material also carries an inherent risk of introduction of exotic pests/ pathogens or their more virulent races into the country. Several pests/ pathogens have been intercepted in transgenics including pests recorded on new host

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during quarantine processing which emphasizes the need for a critical examination of the introduced planting material including transgenics for minimizing the risk of introduction of exotic pathogens or more virulent races for biosecuring India from exotic pathogens (Singh et al. 2002, 2003, Khetarpal et al. 2005, Bhalla et al. 2008).

Materials and Methods

During 2006-2013, a total of 4557 transgenic seed samples of various crops from different countries (Table-1) were received for quarantine processing at the Division of Plant Quarantine, NBPGR, New Delhi. All the seed samples were first examined visually and then under stereo-binocular microscope for the presence of fungal mycelium/ fructifications such as ergot/ sclerotia, rust pustules, smut and bunt balls and symptoms such as discolouration, deformation, malformation and for fungal spores adhered on the seed surface of various crops. The unhealthy-looking seeds of all the crops and leaf/ seed powder were subjected to blotter test. Due to less quantity, three to five seeds of each sample were placed on 3 layers of moist blotters in plastic petri plates and incubated for 7 days at 22 ± 1^{0}C under alternating cycles of 12 hr light and darkness. Observations for the associated pathogenic fungi and bacteria were recorded on the 8^{th} day under stereo-binocular microscope. Slides were prepared and observed under compound microscope for identification of fungi.

All the samples of *Oryza sativa* were subjected to mandatory prophylactic hot water treatment at 52^{0}C for 30 minutes against bacteria and nematodes while infected samples of other crops were salvaged by seed treatment with a mixture of fungicides- Bavistin (0.5%) + Dithane M-45 (1.25%). The pathogenic fungi intercepted in transgenics introduced during this period are presented and their quarantine significance is discussed in the paper.

Results and Discussion

During quarantine processing of 4557 samples of transgenic crops, a total of 740 samples were found contaminated/ infected with various fungi from different countries as listed in Table 1. The fungi associated with these transgenics were identified based on morphological characteristics as *Alternaria padwickii*, *Bipolaris maydis, B. oryzae, B. sorokiniana, Fusarium oxysporum* f. sp. *vasinfectum* and *F. verticillioides* as described by Mathur and Kongsdal (2003) *Phoma sorghina* (Ahmed & Ravinder Reddy (1993) and *Rhizoctonia solani* (Bertus, 1927), Akino & Ogoshi (1995) (Table 2). Among the fungi intercepted, some are of quarantine significance such as:

***Alternaria padwickii* (Ganguly) M.B. Ellis**, the causal agent of stack burn disease of rice was intercepted in *O. sativa* (1 out of 24 samples) from China. Singh et al. (2001) reported that up to 25% of seeds harvested from naturally infected paddy cv. Pusa 33 exhibited the disease in Karnal, India, in 1996.

***Bipolaris maydis* (Nisikado & Miyake) Shoem. [=*Cochliobolus heterostrophus* (Drechsler) Drechsler]**, which causes Maydis leaf blight, Southern leaf blight of maize was intercepted in one out of 93 samples of *Z. mays* from Philippines. *B. maydis* is known to have three races viz., race O, T and C. Race T and C have been reported to be pathogenic only to maize germplasm with Texas male-sterile cytoplasm T and cytoplasm male-sterile C, respectively (http://maizedoctor.cimmyt.org/index.php ?id=251&option=com_content&task=view). Race O infects most maize plants with normal cytoplasm (N) and is prevalent in India. Race T is not reported from India; however, Ram Nath et al. (1973) intercepted it on sorghum germplasm from USA during quarantine processing. In 1970s an epidemic was caused by race T in corn with Texas Male Sterile cytoplasm in most corn -growing areas of the USA which resulted in losses estimated at USD 1 billion (Ullstrup 1972). Sharma & Rai (2000) reported that Maydis leaf blight caused crop losses in productivity as high as 41% in India. Yield losses as high as 68% have been recorded due to *B. maydis* in Cameroon (http://maizedoctor.cimmyt.org/component/content/article/251-maydis-leaf-blight-extended information).

***Bipolaris oryzae* (Breda de Haan) Shoemaker [=*Cochliobolus miyabeanus* (Ito & Kurib.) Drechsler ex Dastur]**, brown leaf spot fungus of rice was intercepted in *O. sativa* from Belgium (in four out of 1784 samples) and USA (two out of 465). *B. oryzae* has been known to cause considerable yield losses. In Nigeria, Aluko (1975) reported 30–40% yield loss due to its severe infection. The Great Bengal Famine of 1943 in India was mainly attributed to brown leaf spot disease, which resulted in yield losses ranging from 40 to 90 % resulting in death of thousands of people (Padmanabhan 1973). Chakrabarti (2001) also reported that yield losses ranged from 26-52% due to brown leaf spot disease in India.

***Bipolaris sorokiniana* (Sorokin) Shoemaker [=*Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur]**, black point, brown spot, leaf-spot blotch and seedling blight fungus was intercepted in one sample of rice from Belgium. Crop losses up to 57% have been observed due to spot blotch infection in wheat from Bolivia (CPC 2007). Damage due to *B. sorokiniana* in the barley production was estimated to range from 30% to 70% of yield losses (Karov et al. 2009). Duveiller et al. (1998) reported yield losses between 20-80% in wheat due to this fungus.

***Fusarium oxysporum* f.sp. *vasinfectum* (G.F. Atk.) Snyder & Hansen** a causal agent of vascular wilt in cotton, was intercepted in one sample of *Gossypium hirsutum* from USA. The pathogen is of great economic importance as it causes substantial crop losses in most of the major cotton-producing areas of the world. Fusarium wilt is a destructive disease of cotton (*Gossypium* spp.) in many countries of the world including Australia, USA, Egypt, Tanzania, China, India, California, Sudan, Israel and Brazil (Davis et al. 2006). Blasingame et al. (2008) also reported that the disease is responsible for losses of 20 million each year across the cotton belt of the USA. Eight races and high genetic diversity have been reported in this fungus throughout the world (Kim et al. 2005, Abo et al. 2005). Therefore, there is high risk of introduction of a new or more virulent race in the country, which may cause severe losses to cotton crop.

***Fusarium verticillioides* (Sacc.) Nirenberg (Syn. *Fusarium moniliforme* Sheldon) [=*Gibberella fujikuroi* (Sawada) S. Ito]**, causal agent of Bakanae disease of rice, stalk/stem/ear rots of maize was intercepted in *Arabidopsis thaliana* from USA (in 17 out of 912 samples); in *Brassica* spp. from USA (1/5); in *Gossypium hirsutum* from USA (55/270); in *Lycopersicon* (*Solanum*) *esculentum* from USA (1/5); in *O. sativa* from Australia (5/14), Belgium (291/1784), China (13/24), France (11/254), Philippines (8/46), UK (1/5) and USA (95/465); in *Z. mays* from Brazil (2/4), Philippines (15/93), Puerto Rico (in one out of one), South Africa (11/17) and USA (193/413). Nuque et al. (1981) reported the existence of race groups of *F. moniliforme* in the Philippines. In 1993 in Karnal, Haryana, India, bakanae incidences of 4.17 and 96.25% caused grain losses of 3.04 and 95.45%, respectively, on inoculated rice cultivar Taraori Basmati (Sunder et al. 1997). Saremi et al. (2008) reported yield losses up to 75% in some fields in Zanjan province of Iran due to the root rot disease in rice. In Bangladesh, Hossain et al. (2013) reported 51.53 and 37.60% yield loss due to bakane disease in the infected hill over its adjacent healthy hill in Aus and Aman seasons, respectively.

***Phoma sorghina* (Sacc.) Boerema Dorenbosch & van Kest. (Syn. *Phoma insidiosa* Tassi)** causing leaf spot of sorghum, maize, and glume blight in rice was intercepted in *O. sativa* from Belgium (12/1784) and USA (3/465) and in *Arabidopsis thaliana* from USA (2/912). Karmakar & Panja (2001) also reported that yield losses due to the glume blight in different rice cultivars ranged from 30-85% during 1997 in West Bengal, India. Interception of *Phoma sorghina* on transgenic *A. thaliana* is a new host record (Singh et al. 2014).

***Rhizoctonia solani* (=*Thanatephorus cucumeris* (Frank) Donk** causal agent of rice sheath blight, basal rot, rhizoctonia head rot, rhizoctonia leaf blight, root rot etc. in wide range of crops world over, was intercepted in one sample each of *O. sativa* from Belgium (1/1784), Philippines (1/46), and

USA (1/465) and *Zea mays* from USA (1/413). *Rhizoctonia solani* can cause disease on at least 200 different plant species around the world with diverse symptoms (Anderson 1982; Salazar et al. 2000). Groth (2008) reported that each year, the sheath blight caused up to 50% decrease in the rice yield under favourable conditions around the world. Yield losses due to sheath blight in India, Philippines and USA ranged from 5.2 to 50% (Jayarajan et al. 2009). Chien & Chung (1963) reported six physiological races in *R. solani* from rice. Banerjee et al. (2012) also reported high genetic variability in the population in different epidemiological regions of West Bengal (India).

Quarantine processing of imported seeds of different transgenic crops resulted in the interception of various pathogens of quarantine/economic significance and new host record. In case those pathogens get introduced into the country, they could have caused serious crop losses. Thus severe losses caused by introduction of these serious pests have been averted due to stringent quarantine processing. Hence, interceptions of pathogens and salvaging of the infected material are of paramount importance to release pest-free material for biosecurity in the country.

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Table 1: Pathogenic fungi intercepted in introduced transgenics during 2006-2013.

S.No.	Host/ Crop	Source/ Country	No. of samples introduced	Samples found infected	Fungi intercepted
1.	*Arabidopsis thaliana*	USA	912	17	*Fusarium verticillioides* (=*Gibberella fujikuroi*
				2	*Phoma sorghina*
		Japan	3	-	-
2.	*Brassica* spp.	USA	5	1	*F. verticillioides*
		The Netherlands	214	-	-
3.	*Glycine max*	USA	7** (Seed powder)	-	-
		USA	3** (Leaf powder)	-	-
4.	*Gossypium hirsutum*	China	4	-	-
		Israel	10	-	-
		USA	270	55	*F. verticillioides*
				1	*Fusarium oxysporum* f.sp. *vasinfectum*
5.	*Oryza sativa*	Australia	14	5	*F. verticillioides*
		Belgium	1784	3+1*	*Bipolaris oryzae =Cochliobolus miyabeanus*
				1*	*B. sorokiniana*
				291	*F. verticillioides*
				6+6*	*P. sorghina*
				1*	*Rhizoctonia solani =Thanatephorus cucumeris* (
		China	24	13	*F. verticillioides*
				1	*Alternaria padwickii*
		France	254	11	*F. verticillioides*
		Philippines	46	8	*F. verticillioides*
				1*	*R. solani*
		UK	5	1	*F. verticillioides*
		USA	465	2	*B. oryzae*
				95	*F. verticillioides*
				3	*P. sorghina*
				1	*R. solani*
6.	*Solanum esculentum*	USA	5	1	*F. verticillioides*
7.	*Triticum aestivum*	USA	1	-	-
8.	*Zea mays*	Argentina	1	-	-
		Brazil	4	2	*F. verticillioides*
		Italy	2	-	-

		Philippines	93	1	*Bipolaris maydis*. =*Cochliobolus heterostrophus*
				15	*F. verticillioides*
		Puerto Rico	1	1	*F. verticillioides*
		South Africa	17	11	*F. verticillioides*
		USA	413	193	*F. verticillioides*

*= Mixed infection, No. of samples not included in total infected
**= No. of samples were not seed but processed for quarantine

Table 2: Morphological features of fungi intercepted in introduced transgenic seeds.

Growth characteristics				Genus
Shape/ type	**Septation**	**Dimension**	**Attachment**	
Conidia straight, fusiform and rostrate with long beak	4-5 septate	Length 120-160 µm and width 12-18 µm (at the broadest part)	Conidiophores bearing solitary conidia	*Alternaria padwickii*
Conidia curved, smooth-walled with rounded ends	8-12 distoseptate	Length 100-150 µm and width 12-20 µm (at the broadest part)	Conidiophores medium to long, slender, single bearing conidia arranged in acropleurogenous manner	*Bipolaris maydis*
Conidia curved, fusiform to obvate with tapering to rounded ends	8-10 septate	Length 90-145 µm and width 12-20 µm (at the broadest part)	Conidiophores straight, long and single bearing conidia arranged in acropleurogenous manner	*B. oryzae*
Conidia ellipsoid, mostly straight, thick-walled with rounded ends	Mostly 9-10 distoseptate	Length 60-90 µm and width 18-20 µm (at the broadest part)	Conidiophores erect, short and single bearing 1-6 conidia arranged in acropleurogenous manner	*B. sorokiniana*
Microconidia oval elliptical and macroconidia falcate along with chlamydospores	Microconidia mostly non septate and macroconidia mostly 3-septate	Microconidia 5-8 µm long and 2-3 µm wide; macroconidia mostly 45-50 µm long and 3 µm wide	Microconidia formed in false-heads on monophialides macroconidia in slimy mass	*Fusarium oxysporum* f. sp. *vasinfectum*

Microconidia mostly and macroconidia fusoid with sharply curved pedicellate basal cell	Microconidia mostly non septate and macroconidia mostly 3 septate	Microconidial length 45-50 µm and width1.5-2.0 µm; macroconidia mostly 45-50 µm long and 3 µm width	Microconidia in chains and macroconidia in wet mass	*F. verticillioides*
Profuse mycelium, pycnidia black and shiny with small to long neck	Conidia non-septate, hyaline, globose and gutulate	Conidial dimension ranged from 1.5-2.5 x 4.5x6.5 µm	Pycnidia superficial or on aerial mycelium	*Phoma sorghina*
Only radiating thick mycelium without sclerotia	Formation of septum in the mycelial branch near the point of origin with dolipore septum, constriction of the branch at the origin point.	-	Branching from parent hypha is at right angle and when cultured on medium, sclerotia not differentiated into rind and medulla	*Rhizoctonia solani*