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Interception of *Peronospora manshurica* in soybean germplasm imported during 1976–2005

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Quarantine processing of 20,108 soybean germplasm samples received during 1976–2005 resulted in the interception of *Peronospora manshurica*, the downy mildew fungus in 1994 samples (9.91%) from 16 countries, including Malaysia and Indonesia, from where it has not been reported. The interception has great quarantine significance in view of the fact that this fungus has not yet been reported from India, is destructive in nature, its oospores can survive for several years in seed and soil, a large number of physiological races exist and zero tolerance limit is prescribed for quarantine purposes. Country-wise details on the introduction of germplasm and interception of downy mildew are presented.

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Keywords: Germplasm, interception, quarantine, *Peronospora manshurica*, soybean.

GLOBALLY there are several instances of plant disease epidemics due to movement of infected seed/other planting materials. Soybean, *Glycine max* (L.) Merrill having both protein and oil, is an important source of low-cost food. The importance of soybean in India has become overwhelming in view of the shortage of edible oil in the country. At present, there is a large area under soybean cultivation in India, particularly in Bihar, Gujarat, Himachal Pradesh, Madhya Pradesh, Maharashtra, Karnataka, Rajasthan and Uttar Pradesh. During 2004–05, the total area under cultivation in India was 7.2 m ha and total production was 6.5 million metric tons with an average yield of 0.76 mt/ha (ref. 1). India occupies 7.14% of total world area and only 2.37% of total world production; the average yield is about one-third of world average.

A large number of soybean germplasm is being introduced in the country for crop-improvement programmes. A number of pathogenic fungi were intercepted during quarantine processing of imported soybean germplasm[2]. Among the interceptions, the most important one is downy mildew of soybean caused by *Peronospora manshurica* (Naum.) Syd., as this mildew is widely distributed the world over, including several countries in Asia but has not yet been reported from India as well as South Asia[3,4]. *P. manshurica* has been repeatedly intercepted on soybean seeds imported from several countries[5]; interceptions from Malaysia[6] and Indonesia[7] were of special significance, as it has not been reported from these countries as well. Johnson and Lefebvre[8] were the first to describe oospore-encrusted soybean seeds and concluded that the disease is seed-borne. Seed transmission was established by Jones and Torrie[9] in 1946.

During the last 30 years (1976–2005), a total of 20,108 seed samples of soybean germplasm were received for quarantine clearance. Country-wise introduction of samples and detection of *P. manshurica* are presented in Table 1. Seed samples were first examined under a stereoscopic binocular microscope for the presence of crust of oospores of downy mildew. Seed samples found treated with *Rhizobium* culture or found free by initial stereo binocular observations, were subjected to washing test. Seeds were stirred in water in a test tube and the suspension was observed in petri plates under stereo binocular microscope. Seed suspensions free from oospores were subjected to centrifugation at 5000 rpm for 10 min; pellets were obtained and examined under compound microscope for the presence of oospores. Seed samples found free by visual inspection/washing test, were randomly tested by staining the teased seed coat with 2,3,5-triphenyltetrazolium chloride (TTC).

Some of the seed samples under stereoscopic microscope showed dull, milky white to light brown crusts on the seed surface. Crusts at higher magnification (50X) showed a

Table 1. Interception of *Peronospora manshurica* in soybean germplasm imported during 1976–2005

Country/source	1976–85	1986–95	1996–2005	Total samples
Belgium	–	3 (1)*	–	3 (1)
Brazil	3 (1)	51 (7)	64(40)	118 (48)
Indonesia	–	11 (1)	32	43 (1)
Israel	–	9 (2)	–	9 (2)
Italy	–	21 (1)	–	21 (1)
Japan	19	4 (2)	16(1)	39 (3)
Malaysia	6 (6)	–	–	6 (6)
North Korea	1 (1)	–	45(12)	46 (13)
Poland	9	3 (2)	–	12 (2)
Russia	–	13 (5)	17	30 (5)
South Korea	–	11 (5)	–	11 (5)
Taiwan	1644 (105)	943 (60)	1469(24)	4056 (189)
Thailand	–	–	9(3)	9 (3)
USA	7072 (1252)	587 (51)	3972(406)	11631 (1709)
USSR	15	75 (3)	–	90 (3)
Zimbabwe	–	206 (3)	–	206 (3)
Other countries	759	2786	233	3778
Total	9528 (1365)	4723 (143)	5857(486)	20108 (1994)
Chi-square test value	267.90	144.40	119.63	719.45

*Figures outside parentheses indicate total number of samples received and those within parentheses indicate infected samples.

honeycomb-like appearance. Seeds partly or completely encrusted with oospores often appeared dull white and had cracks in the seed coat. Such crusts, when examined under compound microscope, revealed masses of hyaline to light brown, thick and smooth-walled oospores measuring 32–42.5 μm in diameter. The oospore measurements and other characteristics agreed with those of *P. manshurica*[10]. Washing test also revealed the presence of few oospores typical of the fungus; however, staining of seed coat did not increase the number of infected samples in a consignment.

Out of 20,108 seed samples of soybean germplasm introduced, 1994 (9.91%) were found to carry *P. manshurica*. It was observed that the highest import, i.e. 11,631 samples (57.84% of total samples introduced) was made from USA followed by 4056 from Taiwan (20.17%), while a limited number of samples were introduced from the rest of the countries. The highest number of samples found infected was also from USA, i.e. 1709 (14.69% of total samples imported from USA) followed by Taiwan with 189 (4.65%). *P. manshurica* was generally intercepted on *G. max*, but it was also intercepted on *G. soja* from USA[11]. A perusal of the literature revealed that a rapid and specific detection method based on polymerase chain reaction was developed by Lai *et al.*[12] for identification of *P. manshurica* and the same can be used for detecting a very low level of infection.

A comparison of data on interceptions of *P. manshurica* during 1976–85 (first decade), 1986–95 (second decade) and 1996–2005 (third decade) was made. It is interesting to note that the per cent infection in samples from Taiwan was 6.38, 6.36 and 1.63 in the first, second and third decade, respectively. This shows a decrease in infection level over the years. However, it was 17.70, 8.68 and 10.22% in samples from USA in the first, second and third decade, respectively, which shows an uneven trend. If we consider the scenario of interception by taking into account samples from all countries together, it was 14.32, 3.02 and 8.29% in the first, second and third decade respectively; the trend similar to that exhibited by samples from USA, which constitutes 57.84% of total samples introduced. The decrease in per cent samples infected in the second decade might be due to the decrease in field incidence of the downy mildew during seed multiplication of the germplasm in those years. The chi-square test values were 267.90, 144.40 and 119.63 for first, second and third decade respectively, and 719.45 for the three decades combined. The observed value of chi-square is greater than that at 1% level of significance for two degrees of freedom, and is, therefore, significant. This proves that the countries differ significantly in their infection to the disease in different yearly strata from 1976 to 2005 and no systematic relationship has been observed with the number of infected samples over the years from a country.

A large number of physiological races are reported in *P. manshurica*. Sinclair and Backman[13] reported that there are 32 known races in *P. manshurica*. In Poland, 11 races were characterized during 1980–85, seven of which were described for the first time (designated races 34–40). A trend towards the appearance of races infecting more cultivars and increasing race variability was recorded; race 35 was the most pathogenic, infecting 12 of the 16 differential cultivars[14]. Li *et al.*[15] reported three new races from ten samples collected from nine counties in China and designated them as Zong 1, 2, and 3. Hartman *et al.*[16] identified 25 physiological races on the basis of disease reactions of different cultivars in USA.

Oospores over-seasoned in plant debris or as seed encrustation constitute the primary source of inoculum, and conidia are responsible for the secondary spread in the field. In addition to the presence of oospores on the surface of the seed coat, mycelium may also invade the seed coat but not the embryonic tissues. Further evidence for seed coat being the primary location of the pathogen was obtained when no infection occurred in seedlings grown from seeds with seed coats removed, while 6.4% of those grown from intact-encrusted seeds was infected[17]. A growth chamber study[18] showed that *P. manshurica* was transmitted from oospore-encrusted seeds at a rate of 16% at a temperature of 15°C, 1% at 20°C , and 0% at 25°C. Oospores of *P. manshurica* could remain viable for eight years[19].

P. manshurica is a quarantine pest for India (a pest of potential economic importance to the area endangered and not yet present there, or present but not widely distributed and being officially controlled). The international standard for tolerance is zero for a quarantine pest. All the infected samples were rejected and incinerated.

The Plant Quarantine (Regulation of Import into India) Order 2003 states that seeds of soybean for sowing purposes should have additional declaration for this downy mildew in the Phytosanitary Certificate (PC) and the special conditions for import should be met, i.e. (i) seed should be free from soil, (ii) commercial import of seeds should have prior approval of the Department of Agriculture and Cooperation. The special condition for import of seed for consumption/processing is as follows: devitalization of seed at the country of origin just prior to export and the particulars of treatment to be endorsed on Phytosanitary Certificate.

Wrather *et al.*[20] estimated that yield losses due to diseases in soybean from ten countries were 14.99 m mt, valued at US $3.31 billion. They further reported that total loss due to downy mildew (*P. manshurica*) in these countries was 387,900 metric tons (mt) (Argentina, 12,300 mt; Canada, 500 mt; China, 363,800 mt; Italy, 1000 mt and USA, 10,500 mt). Crop losses of 10% have been reported from Romania[21].

There are new records that suggest that soybean pests are moving from one area/region to another. Signoret *et al.*[22] reported *P. manshurica* from France, and Zad[23] from Iran. Soybean rust, *Phakopsora pachyrhizi* was first reported from Argentina[24] in 2003.

Likewise, there are several new regional records of soybean pests in different parts of USA, viz. *Fusarium solani* f. sp. *glycines* (sudden death syndrome) in Minnesota[25], *Bean pod mottle virus* in soybean in Alabama[26], *Soybean dwarf virus* in Wisconsin[27], and *Cercospora sojina* (frog-eye leaf spot) in Wisconsin[28]. Records from India include *P. pachyrhizi* in Rajasthan[29] and Chhattisgarh[30].

In view of the interception of *P. manshurica* (a quarantine pest for India) in imported soybean germplasm from 16 countries during the last 30 years, including Malaysia and Indonesia, the countries where it has not been reported, has very high quarantine significance. It is therefore, mandatory to pay attention to prescribed regulations to avoid the introduction of this quarantine pest into the country.

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Forecasting of seasonal monsoon rainfall at subdivision level

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It is shown that time series data of monsoon seasonal rainfall at subdivision level is decomposable into six uncorrelated components. These narrowband processes called intrinsic mode functions, in decreasing order of importance, reflect the influence of ENSO, sunspot activity and tidal cycle on inter annual rainfall variability. The decomposition helps in proposing a statistical method to forecast monsoon rainfall in the three subdivisions of Karnataka.

Keywords: Forecasting, intrinsic mode functions, rainfall, subdivisions.

INDIAN rainfall data are available at two spatial scales in the archives of IITM, Pune (www.tropmet.res.in). The country is considered to be consisting of 33 subdivisions for reporting the monthly rainfall data for the period 1871–2004. This is the smaller spatial scale database. Another database, consisting of the monthly rainfall at the larger spatial scale of eight regions including one at all-India level, is also made available. Recently, we have proposed[1] an approach to analyse and forecast monsoon rainfall data of the regional and all-India time series. The method is based on decomposing the data into empirical modes called Intrinsic Mode Functions (IMFs). The regional-level data have a coefficient of variation defined as the ratio of standard deviation to climatic average (σ/m), ranging from 10 to 18%. The efficiency of the IMF model in one-step-ahead forecasting is about 80%. Thus, the model is able to capture the most important inter-annual variability signatures on the larger spatial scale. A known property of rainfall data is that on larger scales, the variability tends to decrease due to smoothening or averaging effects. Thus, subdivision-level data will show higher variability in comparison with regional data. Since IMF model decomposes the time series into basic uncorrelated empirical modes, one would expect the approach to be qualitatively valid at any scale. However, the forecasting skill will depend on how best the temporal patterns of the signatures are translated into the decomposed modes. The present investigation is aimed at studying the basic modes present in subdivision-level rainfall data, with a view towards forecasting the amount of rainfall.

Rainfall data are available for 33 subdivisions (SD), which make up the geographical extent of the country. The data have been extensively studied for understanding spatial and inter-annual variability (IAV) of the monsoon[2–5]. Also, the data have been used to study the relationship between rainfall and other atmospheric processes such as quasi-biennial oscillation (QBO)[6], Southern oscillation index (SOI)[7] and sunspot index[8]. In spite of the existence of long-term variability or memory signatures, quantitative forecasting of rainfall has remained a daunting task[9]. Here, seasonal (June–September) data of three subdivisions are selected for further study. These are SDs 31, 32 and 33 covering the State of Karnataka (Figure 1). This selection is based on previous studies[10] on the variability structure of station-level rainfall in Karnataka. It was found that broadly the State comprises of three homogenous regions nearly overlapping with the three IMD subdivisions. It was also found that coastal Karnataka (SD-31) has a significant transition probability structure from June to July. There is a tendency for below-normal June rainfall to be compensated by above-normal rainfall in July. Parthasarathy and Pant[7] have shown that rainfall in the above subdivisions is well correlated with QBO and that the data are significantly correlated at 14-year lag. The basic statistics of the data considered here is given in Table 1.

Monsoon rainfall evolves in a random fashion around a few central periods. This can be seen by spectral analysis, wavelet or principal component analysis. Recently, it has

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