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Studies on seed borne mycoflora of soybean seeds by physical methods

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Abstract
The present investigation was carried out in the Department of Plant Pathology, College of Agriculture, I.G.K.V., Raipur (C.G) during the year 2020-21. Six varieties of soybean seed samples *viz*.JS 95-60, JS 97-52, RSC 10-46, RSC 10-71, CG Soya-1 and local variety were selected for experiment. Seed borne fungi can cause distortions, discoloration, spotting, shrinking and disease of seeds which were visible by the naked eyes which were directly concern to the farmers. Categorized the seeds on healthy seeds, discolored seeds, undersized seeds, damaged seeds, plant parts, inert matter, weed seeds, other crop seeds, fraction such as soil, sand, stones, malformations and fungal bodies etc.
Among the studied, RSC 10-46 variety had maximum purity as compared to other soybean varieties while the local variety showed minimum purity. Washing test was also performed. the maximum spore load was found in the seed lot of local variety i.e., ($20x10^{-2}$) and minimum spore load was found in variety CG Soya-1 ($9 x10^{-2}$).

Keywords: Soybean, Dry seed examination, Washing test, Physical method

Introduction
Among the cultivated oilseed crops soybean (*Glycine max* L.) Merrill) is the world's most important seed legume, which contributes 25% of global edible oil. It is popularly known as 'Golden bean' or 'Miracle bean' and 'Wonder bean' of the 20th century because of its characters and usage. It is the most common oilseed crop grown mostly in the rainy season. Soybean seeds have a greater nutritional value it is a major source of protein and vegetable oil. It contains 40-42% proteins, 20-22% oil, 21% starch, vitamins- A, B, C, D & K beside essential amino acids like lysine (5%) and a small amount of calcium, phosphorous, magnesium and iron (Rao *et al.* 2015) [5]. The quality of seeds is affected by seed borne mycoflora. The attack of plant pathogen is one of the reasons for the low productivity of soybean. Most of the economically important plant pathogen is transported from one region to another through seeds or propagating materials. Seed borne diseases are commonly occurring during storage periods if the seeds are stored in a moist dark place. The pathogens can be found in seeds after or before the germination of seeds. Seed borne disease can be spread through wind, water, insect, agricultural equipment and transportation. Germination and seedling vigour is reduced by seed borne mycoflora of soybean and they can destroy or affected grains during storage them unfit for human consumption. Some seed associated fungi can affect the seedling or plant resulting in productive capacity reduced (Rahman *et al*. 1999) [4]. Seed borne fungi can cause distortions, discolouration, spotting, shrinking and disease of seeds which were visible by the naked eyes which were directly concern to the farmers. Seed fungal flora plays a significant role in determining seed quality and longevity. Hence, an experiment was conducted to studies on seed borne mycoflora of soybean seeds by physical methods.

Materials and Methods
1. Dry seed examination
Dry seed inspection can be applied to detect seed-borne mycoflora when present in the seeds may cause changes in shape and size of the seed. First evaluate the seed sample (200 gm) by naked eyes, followed by the magnifying lens and then examined under a stereo binocular microscope to record observations on the mixture of seeds, healthy seeds, discolored seeds, undersized seeds, damaged seeds, plant parts, inert matter, weed seeds, other crop seeds, fraction such as soil, sand, stones, malformations and fungal bodies etc.

2. Washing test

To detect and identify the spores of fungal flora adhered on the seed surface washing test was performed. Two grams of seed taken from working sample in a test tube with 10 ml of sterile distilled water and with the help of a mechanical shaker, for 10 minutes shake the test tube to remove the adhering parts of associated microbes from the seeds. Suspended spores were concentrated by centrifuging at 3000 rpm for 15 to 20 minutes. The pallets used to make a serial dilution of spore suspension and the suspension were discarded. For the serial dilution of 6 samples of seed, arranged in 18 test tubes in a row on test tube stand and fill all with nine ml of sterilized distilled water individually. Add one ml of stock spore suspension in each test tube containing nine ml of distilled water to give 10^{-1} dilution of fungal spore suspension. Labeled it, mixed thoroughly and then added 1 ml of 10^{-1} dilution to the next test tube containing nine ml of distilled water to give 10^{-2} dilutions in the second dilution. These spore suspensions were spread on the PDA poured plates of each dilution in three replicated plates. These plates were incubated at 25±1 ^{0}C under a 12 hours dark and light cycle with NUV light for the 4-5 days. Observations were taken for the identification of mycoflora with the help of a microscope and expressed in terms of spore load counted by counting colonies of individual mycoflora.

Results and Discussion

1. Dry seed examination

Dry seed examination showed the status of discolored seeds, damaged seeds, healthy seeds, shrunken seeds, small or undersized seeds and inert matter in seed lots. Soybean seeds of different varieties were collected from AICRP on soybean, Department of Genetics and Plant Breeding, IGKV, Raipur and one variety was collected from the farmer's field. These varieties were JS 95-60, JS 97-52, RSC 10-46, RSC 10-71, CG Soya-1 and local variety.

Data presented in table 1 showed that the healthy seeds were highest in variety RSC 10-46 (93.22%) followed by JS 95-60 (89.45%), RSC 10-71 (88.38%), CG Soya-1 (87.61%) and JS 97-52 (80.95%) and lowest was noticed in local variety (75.27%). A considerably higher percentage of damaged/broken seeds were recorded in the seed lot of local variety (3.98%) while it was minimum (0.61%) recorded in JS 95-60 variety. The Maximum percentage of discolored seeds were detected in the seed lot of RSC 10-71 (8.01%) followed by local variety (7.57%) and minimum in RSC 10-46 variety (3.15%). The Maximum percentage of shrunken seeds were detected in the seed lot of local variety (3.85%) followed by JS 97-52 variety (3.55%) and minimum in RSC 10-71 variety (0.52%). Small or undersized seeds were highest found in seed lot of JS 97-52 variety (2.20%), followed by local variety (1.93%) and minimum in RSC 10-46 variety (0.84%). The Percentage of the inert matter was recorded maximum found in local variety (7.40%) followed by JS 97-52 variety (4.95%) and minimum in RSC 10-46 variety (0.68%). Among the studied varieties, RSC 10-46 variety had maximum purity as compared to other soybean varieties while the local variety showed minimum purity. Seed borne fungi can cause distortions, discolouration, spotting, shrinking and disease of seeds which were visible by the naked eyes which were directly concern to the farmers.

Variation in purity standards in general and soybean depends on cropping situation, processing and storage etc. Similarly, Sharma (2001) [7] categorized seed samples of a pea from Jaipur and Udaipur in seven groups for examination of dry seeds and recorded maximum healthy seeds (90.25%) in UD 15 and among all samples except UD 16 contained impurities like sand, stones and plant debris. Hoque *et al.* (2014) [1] also examined lentil seed (BARI Masur-1) in dry seed examination and detected seeds were categorized into healthy seeds, spotted seeds, discolored seeds, unfilled seeds and deformed seeds. The highest healthy seeds were recorded in secure 600 WP treatment (57.00%) and lowest in control (35.50%). Pradhan (2019) [3] also studied the seed samples of five varieties of Indian bean in the form of damaged seeds, discolored seeds, small seeds, weed seeds, inert matters and shrunken seeds for examination of dry seeds. Among all varieties of Indian bean IS-29 showed maximum purity as compared to other varieties of Indian bean which supports the finding of the present study.

Table 1: Seed health evaluation of different varieties of soybean seeds

S.N.	Varieties	Healthy seed (%)	Damaged seed (%)	Discolored seed (%)	Undersized seed (%)	Shrunken seed (%)	Inert matter (%)
1.	RSC 10-46	93.22	1.04	3.15	0.84	1.07	0.68
2.	RSC 10-71	88.38	0.71	8.01	1.30	0.52	1.08
3.	CG Soya-1	87.61	0.71	3.70	1.89	3.11	2.98
4.	JS 97-52	80.95	0.84	7.51	2.20	3.55	4.95
5.	JS 95-60	89.45	0.61	4.24	1.49	3.05	1.16
6.	Local variety	75.27	3.98	7.57	1.93	3.85	7.40

A. Discolored seeds B. Shrunken seeds C. Damaged seeds

A. Inert matter B. Small seeds C. Healthy seeds

Fig 1: Seed health evaluation of different varieties of soybean seeds

2. Washing test

To know the spore load present on soybean seed lots, a washing test was performed and data presented in table 2. The data indicate that a total of 81 spore loads were found associated with 6 varieties of soybean. the maximum spore load was found in the seed lot of local variety i.e., ($20x10^{-2}$) which includes 6 spores of *Aspergillus niger*, 5 spores of *Aspergillus flavus*, 4 spores of *Fusarium* spp., 2 spores of *Rhizopus* spp., 1 spore each of *Cladosporium* spp., *Alternaria* spp. and *Trichoderma* spp. This was followed by seed lot of JS 95-60 ($17x10^{-2}$), RSC 10-71($13x10^{-2}$), JS 97-52 ($12x10^{-2}$), RSC 10-46 ($10 x10^{-2}$) and minimum spore load was found in variety CG Soya-1 ($9 x10^{-2}$).

Overall, predominant mycoflora with the highest spore load were *A. flavus* (19) followed by *A. niger*, *Fusarium* spp. (17), *Cladosporium* spp. (10), *Trichoderma* spp., *Macrophomina* spp. (5), *Rhizopus* spp. (4), *Curvularia* spp. and *Alternaria* spp. (2).

In this method, mycoflora was recorded which adhered to the seed surface. Mycoflora recorded associated with seeds of soybean in the present study were also reported by Ramesh *et al.* (2013) studied seed mycoflora of soybean collected from different locations & other sources were determined by seed washing test. Six seed fungal flora of soybean *viz. Macrophomina phaseolina, Fusarium oxysporum, Aspergillus flavus, Aspergillus niger, Phoma* spp. and *Sclerotinia sclerotiorum* were identified and isolated from seed samples collected from different locations were. Similarly, Trivedi and Rathi (2015) [8] observed the seed mycoflora in chickpea by washing test and reported the presence of spores of, *Fusarium moniliforme, F. oxysporum* and *Aspergillus* spp. in seed washing of chickpea. Kesharwani (2018) [2] also performed a washing test to examine the presence of spore load on pea. The highest spore load was recorded from the seed sample of the local variety i.e. ($24x10^{2}$) and the lowest spore load was recorded in the Shubhra variety ($3x10^{2}$) support the findings of the present study.

Table 2: Efficacy of washing test to detect the mycoflora associated with soybean seeds.

S.N.	Varieties	NUMBER OF CFU ($x10^{-2}$)									Total CFU($x10^{-2}$)
		A. niger	*A. flavus*	*Rhizopus* spp.	*Cladosporium* spp.	*Alternaria* spp.	*Macrophomina* spp.	*Fusarium* spp.	*Curvularia* spp.	*Trichoderma* spp.	
1.	RSC 10-46	1	2	-	1	-	2	3	1	-	10.0
2	RSC 10-71	2	3	-	-	1	-	5	-	2	13.0
3.	CG Soya-1	-	4	1	1	-	-	2	1	-	9.0
4.	JS 97-52	2	3	1	2	-	-	2	-	2	12.0
5.	JS 95-60	6	2	-	5	-	3	1	-	-	17.0
6.	Local variety	6	5	2	1	1	-	4	-	1	20.0
Total mycoflora		17	19	4	10	2	5	17	2	5	81.0

References

1. Hoque MA, Ali MA, Haque Mahfuzul AHM, Mehraj H, Uddin Jamal AFM. Efficacy of some fungicides for the improvement of seed quality in lentil. Int. J Sustain. Crop Prod. 2014;9(3):22-26.
2. Kesharwani A. Seed health evaluation of different Pea varieties (*Pisum sativum* L.). M.Sc. (Ag) Thesis submitted to Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). 2018.
3. Pradhan K. Seed health evaluation of different varieties of Indian bean (*Lablab purpureus* L.). M.Sc. (Ag.) Thesis submitted to Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). 2019.
4. Rahman S, Vearasilper S, Srichuwong S. Detection of Seed-Borne Fungi in Mungbean and Blackgram Seeds. Sustainable technology development in crop production, 1999, 1-3.
5. Rao TV, Rajeswari B, Keshavulu K, Varma VS. Studies on seed borne fungi of soybean. SSRG J Agric Env Sci. 2015;2(1):16-24.
6. Sahu D. Studied seed health evaluation of different varieties of Lentil (*Lens culinaris* M.). M.Sc. (Ag) Thesis submitted to Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). 2020.
7. Sharma PKS. Micro-organism Associated with Seeds of Pea (*Pisum sativum* L.), Their Pathogenic Potential and Disease Management. Ph.D. Thesis submitted to Maharana Pratap University of Agriculture and Technology, Udaipur (Raj.). 2001.
8. Trivedi L, Rathi YPS. Detection of seed mycoflora from chickpea wilt complex seedborne *Fusarium oxysporum f. sp. ciceri* diseased seeds. World Journal of Pharmacy and Pharmaceutical Sciences (WJPPS). 2015;4(9):1242-1249.