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RESEARCH PAPER

Evaluation of different seed health testing methods for seed borne fungal diseases of sorghum

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Abstract: Among the several diseases affecting sorghum, majority of them are known to be seed-borne. Seed health testing of different sorghum seed samples collected from different sources in Dharwad, revealed the pre-dominance of pathogenic fungi *viz., Colletotrichum graminicola* causing anthracnose, *Exserohilum turcicum* causing turcicum blight and *Curvularia lunata* causing leaf spots on sorghum. Seed samples also revealed the presence of fungi like *Aspergillus* spp. which are known to produce deadly afla-toxins which is gaining importance in the context of post GATT-WTO era. *Colletotrichum graminicola* (11.98%) *Exserohilum turcicum* (11.53%) and *Curvularia lunata* (10.40%) were the most predominant fungi encountered. Seed samples collected from farmers source exhibited maximum infection of *Colletotrichum graminicola* and *Exserohilum turcicum*. Among the different seed health testing methods tested, Deep freezing Blotter Method was found to be most efficient for the quick and accurate diagnosis of *Curvularia lunata*, *Alternaria alternata* and *Colletotrichum graminicola* and for *Exserohilum turcicum,* 2, 4-D blotter method was found suitable.

Key words: Pathogenic, Seed borne, Seed health, Sorghum

Introduction

Sorghum (*Sorghum vulgare* Pers.) is the fifth most important cereal crop in the world after wheat, rice, maize and barley. It is found in the arid and semi-arid parts of the world, due to its feature of being extremely drought tolerant. The nutritional value of sorghum is same as that of corn and that is why it is gaining importance as livestock feed. Sorghum is also used for ethanol production, producing grain alcohol, starch production, production of adhesives and paper other than being used as food and feed. The crop in the country stands at the third place in context of importance after wheat and rice. The grain had been used for consumption of both humans and livestock, more than 35 per cent of sorghum is grown directly for human consumption and the rest is used primarily for animal feed and forage, alcohol production and industrial products (Awika and Rooney, 2004). In India the total area under sorghum is 6.07 million ha with production of 5.24 million tonnes and productivity of 697 kg/ha. Maharashtra, Karnataka, Andhra Pradesh, Madhya Pradesh and Tamil Nadu are the five sorghum producing states which together account for about 82 per cent of the area and more than 83 per cent of the total production. In Karnataka area under sorghum is 1104 thousand hectares with the production of 955 thousand tonnes and productivity is 865 kg/ha (Anon., 2016)

About 50 diseases are noticed in sorghum, but only 30 of them are found in India (Patil *et al*., 2011). Among these diseases affecting sorghum, majority of them are known to be seed-borne *viz.,* turcicum blight caused by *Exserohilum turcicum*, head blight caused by *Fusarium moniliforme*, anthracnose caused by *Colletotrichum graminicola,* zonate leaf spot caused by *Gloeocercospora sorghi,* downy mildew caused by *Peronosclerospora sorghi,* leaf spot and blight caused by *Phoma sorghina,* grain smut, loose smut and head smut caused by *Sporisorium sorghi*, *Sporisorium cruentum*, *Sporisorium reilianum,* respectively. Many of the diseases that cause reduced yields in sorghum have seed-borne phases. Seed borne inoculum therefore, has severe implications for yield, seed production and distribution systems, trade, human nutrition and germplasm. The management of these pathogens during the seed-borne phase is considered to be the cheapest disease control strategy (Shenge, 2007).

The present investigation was carried out during the year 2017-18 to know the current status of different seed-borne fungal pathogens of sorghum in Dharwad and to evaluate the different seed health testing methods for quick and accurate detection of seed-borne fungi.

Material and methods

Sorghum seed samples were collected from different sources in Dharwad *viz.,* AICRP centers on sorghum, from farmers and seed companies. Collected untreated seed samples were stored at room temperature (25±2°C) and further they were subjected to initial seed health testing by Standard Blotter Method (Anon., 1999). Four hundred seeds of each variety obtained from different locations were tested by employing standard blotter method with three replications. Three pieces of blotting paper of 90 mm size were moistened with distilled water and placed in 90 mm sterilized petriplates after draining excess water. Untreated seeds were placed at the rate of twenty seeds per petriplate at equal distance in each petriplate. The plates were incubated at room temperature (25±2°C) under alternate cycles of 12 hours Near Ultra Violet light and darkness. After 7 days of incubation, the seeds were examined under stereoscopic binocular microscope for the associated fungi (Khare, 1996). Different seed health testing methods *viz.,* Standard blotter method, deep freezing blotter method, 2, 4-D blotter method, water agar method, agar plate method with potato dextrose agar were

evaluated for the detection of major seed-borne fungal infections in sorghum. Deep freezing blotter method is similar to standard blotter method but the petriplates are incubated at 25±2°C for first 24 hrs under alternate cycles of 12 h NUV light and darkness, for next 24 hrs the plates are incubated at -20°C and then kept back under original conditions for next 6 days. In 2,4-D blotter method, 20 seeds per Petriplate are placed on moistened blotter dipped in 0.2 per cent solution of sodium salt of 2,4-dichlorophenoxy acetic acid and Petriplates were incubated in the same way as described under standard blotter method. In water agar method seeds were placed at the rate of 10 seeds per Petriplate containing 20 ml of 2 per cent water agar. The Petriplates were incubated for 7 days as described under standard blotter method. In agar plate method with Potato Dextrose Agar, surface sterilized seeds were placed at the rate of 10 seeds per Petriplate containing 20 ml of PDA and petriplates were incubated for 7 days as described under standard blotter method.

Results and discussion

The seed samples of sorghum collected from different sources such as AICRP on Sorghum, farmers and seed companies were tested initially by employing standard blotter method and the results are presented in Table 1. Totally six fungi including both saprophytic as well as pathogenic were encountered. The results of this study indicated the dominance of *Colletotrichum graminicola* (11.98 %), *Exserohilum turcicum* (11.53 %), and *Curvularia lunata* (10.40 %). Other saprophytic fungi included species of *Aspergillus* (33.97 %), *Alternaria alternata* (22.33 %) and the species of *Cercospora*

Table 1. Seed health testing of different sorghum cultivars collected from Dharwad district by standard blotter method

Source	Cultivar	Per cent seed infection						
		Cercospora sorghi	*Alternaria alternata*	*Exserohilum turcicum*	*Colletotrichum graminicola*	*Curvular ialunata*	*Aspergillus* spp.	Total
AICRP	SPV 2217	0.00	15.00	0.00	20.00	0.00	5.00	40.00
Sorghum	Kodmurky	3.00	0.00	7.00	0.00	5.00	2.00	17.00
	P-Chitra	25.00	25.00	10.00	0.00	14.00	5.75	79.75
	IS-8607	0.00	3.75	0.00	6.25	3.25	10.00	23.25
	SPV-1829	0.00	5.00	5.50	4.50	0.00	20.00	35.00
	CSV-216R	4.00	1.00	0.00	0.00	7.00	3.75	15.75
	M-35-1	0.00	10.00	7.00	5.00	0.00	14.00	36.00
	Barsi Jawar	0.00	0.00	2.00	2.00	4.00	12.50	20.50
	Muguti-5-4-1	0.00	2.25	2.25	0.00	0.00	0.00	4.50
	Phule Moti	5.00	8.00	0.00	0.00	5.00	5.00	23.00
	Jalna Dagdi	0.00	1.25	0.00	0.00	3.00	15.00	19.25
	PVK Kranti	10.50	0.00	1.75	7.00	6.50	4.00	29.75
	SPV-462	0.00	2.50	0.00	0.00	5.00	12.50	20.00
	Annigere	0.00	7.00	12.00	0.00	0.00	0.00	19.00
	Phule Vasudha	4.00	5.00	4.00	0.00	4.50	0.00	17.50
	BJV- 44	8.00	2.00	0.75	12.00	1.25	2.00	26.00
	Phule Jyothi	5.00	5.75	3.75	0.00	3.75	1.50	19.75
	E-36-1	0.00	0.00	0.00	3.00	4.00	15.00	22.00
	SPV-86	0.00	12.50	0.00	0.00	2.00	4.00	18.50
	DSV-4	4.25	8.75	3.50	0.00	4.50	1.50	22.50
	CSV-27	4.50	7.50	0.00	0.00	0.00	8.75	20.75
	IS-2205	0.00	0.00	4.00	10.00	0.00	8.00	22.00
	DSV-6	1.50	4.00	8.00	3.00	1.00	11.25	28.75
	IS 2312	0.00	0.00	0.00	10.00	4.00	8.00	22.00
	SGMRN 12-3-2	0.00	5.00	7.00	3.00	3.00	20.00	38.00
Farmers	M 35-1	0.00	11.00	8.00	9.00	0.00	25.00	53.00
	SPV 2217	0.00	12.00	7.00	6.00	1.00	8.00	34.00
	Jawar 541	0.00	3.75	0.00	5.00	0.00	10.00	18.75
	Bijapur	0.00	7.00	5.00	5.00	0.00	25.00	42.00
Seed Companies	Varada hybrid jowar CSH-16	0.00	3.00	0.00	0.00	0.00	12.00	15.00
	Krishna hybrid jowar	4.00	4.00	0.00	0.00	0.00	15.00	23.00
	Laxmi 369 hybrid jowar	11.00	12.00	0.00	0.00	0.00	30.00	53.00
	Dhana laxmi CSH-14	5.00	26.00	12.00	0.00	17.00	10.00	70.00
	Total	94.75	212.00	109.50	113.75	98.75	322.50	
		9.98%	22.33%	11.53%	11.98%	10.40%	33.97%	

sorghi appeared in traces hence further studies were restricted to *Exserohilum turcicum, Colletotrichum graminicola* and *Curvularia lunata*. The results are in agreement with Mohammed *et al*. (2015) who reported the genera of *Aspergillus, Alternaria, Arthrinium, Bipolaris, Botrytis, Cercosporella, Cladosporium, Colletotrichum, Curvularia, Fusarium, Penicillium, Phoma* and *Trichoderma.* Godbole (1982) reported 21 species on seed samples predominance being of *Curvularia lunata*, *Fusarium moniliforme*, *Drechslera tetramera*, *D. rostrata*, *Alternaria tenuis*, and *Phoma* spp. Panchal and Dhale (2011) tested 24 seed samples, using the blotter and agar plate methods. Twenty eight fungal species were appeared in the seeds of eight different varieties of sorghum, maximum incidence was of *Curvularia lunata*.

In the present investigation, among the seed samples of different cultivars tested from different places, P. Chitra cultivar from AICRP on Sorghum exhibited maximum seed-borne infections compared to other cultivars. In general seed samples from AICRP on Sorghum and farmers exhibited maximum infection of *Colletotrichum graminicola, Exserohilum turcicum* and *Curvularia lunata*. This might be because of provenance effect and microclimatic conditions existing in these areas for which the seeds were exposed during and after harvest. Among the 33 seed samples tested, per cent infection of *Colletotrichum graminicola* varied from 2 to 20 per cent, *Exserohilum turcicum* varied from 0.75 to 12 per cent and *Curvularia lunata* varied from 1 to 17 per cent. Similar results were obtained by Islam *et al.* (2009) who reported that about 36 % of the sorghum seeds were infected by different species of fungi including *Curvularia lunata*, *Bipolaris sorghicola* and *Colletotrichum graminicola*.

Comparative efficacy of different seed health testing methods

The results of the experiment are presented in Table 2. Several criteria are involved in selecting a suitable method for the detection of seed borne fungi. The primary criterion is that the method should be sensitive, simple and reproducible. Anon. (1999) advocated that dry seed examination itself is not an adequate method and is a supplement to the incubation method with some modifications, which are imperative to provide full information regarding the identification of the pathogen. Besides, dry seed examination does not provide information on viability of the seeds. Keeping this in view, a study to compare the efficacy of five routine seed health testing methods in detecting the seed borne fungal infection in sorghum was undertaken. Among the five different methods employed for the detection of seed-borne fungal pathogens of sorghum, deep freezing blotter method was found to be good for the detection of *Curvularia lunata*, *Alternaria adicale* and *Colletotrichum graminicola*. Similar results were obtained by Ishrat and Shahnaz (2009) who reported that deep freezing method was the best method for the detection of *Drechslera* sp., *Colletotrichum graminicola.* And *Penicillium* sp., while agar plate method was suitable for the detection of *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp. And *Rhizopus* sp. From maize seeds. In deep freezing blotter method, examination of seed-borne pathogens is easy as the method discourages seed germination and the germinating adical and plumule will not come in the way of observation like in other methods. Turgay and Unal (2009) investigated that higher number of fungi was isolated by using deep-freezing method as compared to agar and blotter methods.

Limonard (1968) recommended deep freezing blotter method as an alternative method to 2, 4-D blotter method since it is also known to suppress germination of seed.

Conclusion

Deep freezing blotter method can be recommended for routine seed health diagnosis of seed-borne fungal infection in sorghum as it suppress germination, so the dead seed will act as a natural substrate for the growth of fungi and also this method is simple, sensitive and reliable.

Table 2. Evaluation of seed health testing methods in detecting the seed-born fungal infections in sorghum

Sl. No.	Treatment	Per cent seed infection by					
		Curvularia lunata	*Exserohilum turcicum.*	*Colletotrichum graminicola*	*Alternaria alternata*	*Aspergilus* spp.	Total
1	Standard blotter method	0.00(0.00)*	12.00(20.25)	13.00(21.12)	11.00(19.36)	30.00(33.19)	66.00(54.30)
2	Deep freezing blotter method	12.00(20.25)	12.00 (20.25)	16.00(35.26)	17.00(24.34)	15.00(22.77)	72.00(58.02)
3	2,4-D blotter method	10.00(18.42)	16.00(23.56)	11.00(19.36)	13.00(21.12)	0.00(0.00)	50.00(44.98)
4	Water agar method	10.00(18.42)	0.00(0.00)	12.00(20.25)	0.00(0.00)	9.00(17.45)	31.00(33.81)
5	Potato dextrose agar method	12.00(20.25)	10.00(18.42)	13.00(21.12)	12.00(20.25)	13.00(21.12)	60.00(50.74)
	Total	44.00(41.53)	50.0044.98	65.0053.70	53.0046.70	67.0054.91	279.00
	S.Em.±	0.42	0.33	0.55	0.44	0.47	
	C.D. at 1 %	1.88	1.05	1.75	1.40	1.48	

*Figures in parentheses indicates arcsine values

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