Indian Phytopathology (2019) 72:421–426
https://doi.org/10.1007/s42360-019-00146-5

RESEARCH ARTICLE

Pokkah boeng resistance in popular rabi sorghum cultivars and effects of the disease on leaf chlorophyll

I. K. Das[1] · Sunil Kumar[1] · N. Kannababu[1] · Vilas A. Tonapi[1]

Received: 5 December 2018 / Revised: 26 March 2019 / Accepted: 27 May 2019 / Published online: 11 June 2019

Abstract

Field experiments were conducted with nineteen sorghum (*Sorghum bicolor* L. Moench) cultivars that included fifteen rabi varieties, two kharif varieties and two rabi parental lines for evaluation of pokkah boeng (c.o. *Fusarium subglutinans*) resistance. Stem injection method of artificial inoculation was used to develop the disease under field conditions during two successive rabi seasons of 2015 and 2016. Leaf chlorophyll was measured by SPAD-502 chlorophyll meter to compare changes in chlorophyll content in inoculated plants over the control. Disease severity index ranged from 9.5–27.0% in 2015 and 10.4–32.5% in 2016. The cultivars widely varied in pokkah boeng resistance. CSV 19SS was identified as the most resistant among the test cultivars CSV 18R, DSV4, E 36-1 and CSV 8R as moderately resistant and SLR 30, RS 585 and M35-1 as susceptible cultivars. The disease had adverse effects on leaf chlorophyll content at vegetative stage particularly in susceptible cultivar (~21% reduction). This is the first information on the status of pokkah boeng resistance in popular rabi sorghum cultivars, which will help selection of varieties for cultivation.

Keywords Sorghum · Pokkah boeng · Incidence · Severity index · SPAD chlorophyll

Introduction

Pokkah boeng or twisted top disease caused by the fungus *Fusarium subglutinans* (formerly *F. moniliforme* var. *subglutinans*), is an emerging disease of sorghum in India (Das et al. 2011a). It was first reported by Ramakrishnan (1941) from Tamil Nadu. Later Garud et al. (1990) reported the disease on sorghum from Maharashtra. In general, the disease was minor and sporadic in nature. However, since 2009 it is occurring in mild to moderate form almost every year in major sorghum growing regions in India giving an indication of its changing significance. A pokkah boeng infected plant generally develop leaf symptoms that include chlorosis, leaf twisting, top rotting, and occasionally stem symptoms (vertical discoloration on the stem just above the basal node, bending of the stem, horizontal cuts on the basal stem as if made with knife). During rabi season of 2010 up to 35% incidence was recorded on some sorghum genotypes in Andhra Pradesh and there was strong negative influence of the disease on panicle weight ($r = -0.33$, $p = 0.002$) and grain yield ($r = -0.34$ $p = 0.001$) especially under post-flowering moisture stress conditions (Das et al. 2011b). Studies on interrelationship among plant traits revealed that grain yield, plant height and earhead length were affected by the disease (Das et al. 2015).

Pokka boeng resistance of the presently grown sorghum cultivars are not known as they were not screened for the disease. Many recent incidences of the popular cultivars showing severe pokkah boeng are reported from different parts of India and it is becoming almost a regular phenomenon (AICRP-S 2014). There is an urgent need to evaluate them for resistance against pokkah boeng using standard screening technique. In sugarcane, artificial inoculation for development of pokkah boeng is done by soaking of setts or injection of stems with a spore suspension (Siti Nordahliawate et al. 2008). Recently an efficient artificial inoculation method has been developed using stem injection method for assessment of pokkah boeng resistance in sorghum (Das et al. 2015).

In most occasions pokkah boeng in sorghum is initially expressed as leaf chlorosis. Other symptoms like leaf twisting, top rotting, and stem symptoms appear in the subsequent phases. Leaf chlorosis is an indication of reduced

✉ I. K. Das
das@millets.res.in

[1] ICAR-Indian Institute of Millets Research, Hyderabad 500030, India

chlorophyll content, which plays important role in overall growth and development of the plant. Tiancheng et al. (2000) determined chlorophyll content of leaf in rice, cotton, corn, sorghum and soybean using SPAD chlorophyll meter and found good correlations between the SPAD readings and chlorophyll content in the leaves. The SPAD chlorophyll meter reading was significantly related with plant height, total dry matter, panicle weight and grain yield in rabi sorghum (Talwar et al. 2009). Whether sorghum cultivars respond similarly on pokkah boeng induced leaf chlorosis is not known. Such information will help better understand pokkah boeng disease in sorghum. Previous studies have used SPAD chlorophyll meter to measure leaf chlorosis or greenness on many crops including sorghum (Talwar et al. 2009; Yamamoto et al. 2002) and maize (Dwyer et al. 1991). The objective of the present work was to assess popular sorghum cultivars for resistance to pokkah boeng disease and find out the effects of the disease on leaf chlorophyll.

Materials and methods

Plant materials

A total of 19 sorghum cultivars including 15 rabi and 2 kharif varieties and 2 rabi parental lines were used for the present experiment. Detail information on the sorghum cultivars used in the study is given in Table 1. The rabi parental line SLR30 was considered as susceptible check based on earlier studies (Das et al. 2011a, 2015). However, no confirmed resistant check was known so far. Seed materials were obtained from gene bank of Indian Institute of Millet Research, Hyderabad.

Field experiment

Field experiments were conducted in replicated trials at the Indian Institute of Millet Research, Hyderabad, during rabi seasons of 2015 and 2016. Crop was raised during the period between mid-Septembers to mid-January in both the years. Trials were laid out in RBD with three replications. Each cultivar was grown in a plot with two consecutive rows of 4 m length, with 60 cm row spacing and a plant spacing of 10–15 cm within rows. Standard crop management practices were followed to raise the crop. Thinning of the crop was carried out at 20 days after emergence to maintain around 60 plants per plot. Randomly selected 10 plants were tagged and treated as control, which received no inoculation. Remaining plants were inoculated individually by stem injection with aqueous spore suspension (1×10^5 spores/ml) (about 1 ml/plant) of a virulent isolate of *F. subglutinans* (Pb1006) with the help of a hypodermic needle at 30 days after emergence (DAE). Details about inoculum preparation and inculcation method were followed as given in Das et al. (2015).

Disease incidence, severity and rating scale

Based on disease symptoms each plant was scored on a scale of 0–4 (Patil et al. 2007; Das et al. 2015), where 0 = healthy plant with no pokkah boeng symptom, 1 = Leaf chlorosis,

Table 1 Information on the sorghum cultivars used in the study

Cultivar	Sorghum type	Season	Source
CSV 8R	Grain sorghum, variety	Rabi	VN Marathwada Krishi Vidyapeeth, Parbhani
CSV 14R	Grain sorghum, variety	Rabi	Indian Institute of Millets Research, Hyderabad
CSV 216R	Grain sorghum, variety	Rabi	Mahatma Phule Krishi Vidyapeeth, Rahuri
CSV 18R	Grain sorghum, variety	Rabi	VN Marathwada Krishi Vidyapeeth, Parbhani
CSV19SS	Sweet sorghum, variety	Kharif	Mahatma Phule Krishi Vidyapeeth, Rahuri
CSV20	Grain sorghum, variety	Kharif	Indian Institute of Millets Research, Hyderabad
CSV 26R	Grain sorghum, variety	Rabi	Center of Rabi Sorghum, Solapur
CSV 29R	Grain sorghum, variety	Rabi	University of Agricultural Sciences, Bijapur
DSV4	Grain sorghum, variety	Rabi	University of Agricultural Sciences, Dharwad
PVK Kranti	Grain sorghum, variety	Rabi	Panjabrao Deshmukh Krishi Vidyapeeth, Akola
Phule Chitra	Grain sorghum, variety	Rabi	Mahatma Phule Krishi Vidyapeeth, Rahuri
Phule Revati	Grain sorghum, variety	Rabi	Mahatma Phule Krishi Vidyapeeth, Rahuri
Phule Suchitra	Grain sorghum, variety	Rabi	Mahatma Phule Krishi Vidyapeeth, Rahuri
Phule Vashudha	Grain sorghum, variety	Rabi	Mahatma Phule Krishi Vidyapeeth, Rahuri
Parbhani Moti	Grain sorghum, variety	Rabi	VN Marathwaada Krishi Vidyapeeth, Parbhani
M35-1	Grain sorghum, variety	Rabi	Mahatma Phule Krishi Vidyapeeth, Mohl
E 36-1	Grain sorghum, variety	Rabi	Indian Institute of Millets Research, Hyderabad
RS 585	Grain sorghum, R-line	Rabi	Indian Institute of Millets Research, Hyderabad
SLR 30	Grain sorghum, R-line	Rabi	Center of Rabi Sorghum, Solapur

2 = Leaf twisting, 3 = Top rotting, and 4 = Stem symptom. Leaf symptoms were recorded at vegetative (45 DAE) and stem symptoms at reproductive (75 DAE) growth phase. Disease incidence (DI) was calculated as per cent of symptomatic plants. Number of plants with specific score was counted in each plot and disease severity index (DSI) was calculated using the following formula:

$$\mathrm{DSI}(\%) = \frac{\sum \text{Sum of scores for each Plant}}{\text{Maximum score in the scale} \times \text{Number of plants scored}} \times 100$$

A pokkah boeng rating scale for identification of resistance was developed based on severity index (Das et al. 2015). As per this scale DSI < 1.0% = highly resistant (HR), 1–10% = resistant (R), 11–20% = moderately resistance (MR), 21–30 = susceptible (S), and > 30 = highly susceptible (HS).

Measurement of leaf chlorophyll

Leaf chlorophyll was measured by chlorophyll meter SPAD-502 Plus. Randomly selected five inoculated and five control plants in each cultivar were considered for SPAD readings. In each selected plant readings were recorded on three leaves (top three) and three spots per leaf (top, middle and bottom) at 45 DAE. Thus, one mean of forty-five readings (3 spots × 3 leaves × 5 plants) for inoculated and another mean of same number of readings for control were obtained for each cultivar. Change of chlorophyll content in inoculated plant was obtained by deducting its reading from respective control and expressed in percentage.

Statistical analysis

Variance in data for individual year was analyzed separately to test the significance of differences among the traits. The error variances in the field experiment over the years were heterogeneous, as revealed by Bartlett's test (Bartlett, 1937). Therefore, data were not combined and year-wise analysis was performed. Pair-wise comparisons of means were based on Tukey HSD method using software Statistix (version 8.1). Means were compared at 5% level of significance. Correlation coefficients among incidence, severity and SPAD chlorophyll meter readings were estimated using MS Excel.

Results and discussion

Disease incidence

Artificial inoculation of sorghum seedlings with *F. subglutinans* at 30 DAE resulted in development of pokkah boeng under field conditions. Disease incidence varied significantly among the cultivars in both the years (Table 2). Pokkah boeng incidence ranged from 34.5–83.5% (susceptible check, SLR 30 = 74.0%) in 2015 and 26.0–65.0% (SLR 30 = 65.0%) in 2016. Stem injection is an efficient method of pokkah boeng development in sorghum and an incidence of up to 84% was reported previously under artificial inoculation (Das et al. 2015). However, under natural field condition maximum incidence reported was 35% (Das et al. 2011b). Mean incidence was higher in 2015 (64.4%) than 2016 (41.9%). This was because the month of October in 2015 experienced more rainfall as compared to 2016 (data not shown). Humid conditions in October (early growth phase) favored disease development causing more incidences in 2015. Incidences in CSV 19SS and E36-1 in 2015 and in CSV 18R, CSV 19SS and Phule Suchitra in 2016 were significantly less than the mean incidence. The cultivars CSV 8R, CSV 18R, CSV 19SS, CSV 20, DSV 4, PVK Kranti, Phule Suchitra, Phule Vasudha and E 36-1 recorded

Table 2 Response of popular sorghum cultivars to pokkah boeng disease under artificial inoculation in field conditions

Cultivar	Incidence (%)		Severity index (%)	
	2015	2016	2015	2016
CSV 8R	62.0	38.0	17.5	18.5
CSV 14R	70.5	36.0	20.0	21.0
CSV 216R	69.0	35.5	22.5	17.5
CSV 18R	58.5	26.0	15.0	11.5
CSV19SS	34.5	32.5	9.5	10.4
CSV20	62.0	44.0	19.5	23.0
CSV 26R	68.0	43.0	21.5	17.5
CSV 29R	83.5	46.0	27.0	20.0
DSV4	59.0	37.5	17.0	12.5
PVK Kranti	62.5	53.5	19.5	27.0
Phule Chitra	69.5	39.5	21.0	19.5
Phule Revati	66.0	35.5	19.5	21.0
Phule Suchitra	57.5	26.0	17.0	20.4
Phule Vashudha	62.5	40.5	18.5	22.0
Parbhani Moti	79.5	46.5	25.5	23.5
M35-1	81.0	63.5	24.5	29.5
E 36-1	40.5	34.5	10.5	19.0
RS 585	64.5	53.0	24.5	25.0
SLR 30	74.0	65.0	27.0	32.5
Mean	64.4	41.9	19.8	20.6
CD at 5%	11.4	7.9	3.7	4.4
Probability	0.040	0.004	0.005	0.007

significantly less incidence than the susceptible SLR 30 in both the years. Incidence in CSV 14R, CSV 216R, CSV 26R, CSV 29R, Phule Chitra, Phule Revati, Parbhani Moti, M 35-1, RS 585 in 2015 and in M35-1 in 2016 was at par with the susceptible SLR 30.

Disease severity

Pokkah boeng severity, that was an index based on four types of symptoms (phase-1 to phase-4), varied significantly among the cultivars in both the years. Severity ranged from 9.5–27.0% (susceptible check, SLR 30 = 27.0%) in 2015 and 10.4–32.5% (SLR 30 = 32.5%) in 2016. Up to 24.6% severity of was reported previously under artificial inoculation with stem injection of sorghum with *Fusarium subglutinans* (Das et al. 2015). Though mean incidence was higher in 2015 than 2016, mean severity was almost similar in both the years (19.8% in 2015) and (20.6% in 2016) (Table 2). This was because the share of phase-1 symptom (leaf chlorosis) was higher (53.6%) in 2015 than 2016 (16.6%) but that of phase-3 symptom (top rotting) was less (3.2%) in 2015 than 2016 (16.3%) (data not shown). As phase-1 symptom has proportionately less contribution than phase-3 symptom in determining magnitude of severity index, severity did not vary much over the years in spite of higher incidence in 2015. The cultivars CSV 8R, CSV 14R, CSV 216R, CSV 18R, CSV 19SS, CSV 20, CSV 26R, CSV 29R, DSV4, PVK Kranti, Phule Chitra, Phule Revati, Phule Suchitra and Phule Vasudha recorded significantly less severity than the susceptible SLR 30 in both the years. Of these, severity in CSV 18R, CSV 19SS and E 36-1 in 2015 and CSV 18R, CSV 19SS and DSV 4 in 2016 were also significantly less than the mean severity. So cultivars CSV 18R, CSV 19SS, E 36-1and DSV 4 showed better performance against pokkah boeng than others in both the years. Severity in CSV 29, Parbhani Moti, M 35-1, RS 585 in 2015 and in PVK Kranti and M35-1 in 2016 was at par with the susceptible SLR 30. Therefore, cultivars M35-1 and SLR 30 were considered poor performer against pokkah boeng across the years. The finding was consistent with previous studies and SLR 30 was known for pokkah boeng susceptibility (Das et al. 2011a, 2015).

Resistance in cultivar

Base on disease reactions under artificial inoculation, out of 19 varieties one was resistant (CSV 19SS), 10 moderately resistant (E 36-1, CSV 18R, DSV4, Phule Suchitra, CSV 8R, Phule Vasudha, CSV 20, PVK Kranti, Phule Revati and CSV 14R) and 8 were susceptible (Phule Chitra, CSV 26R, CSV 216R, M35-1, RS 585, Parbhani Moti, CSV 29R and SLR 30) to pokkah boeng in 2015 (Table 2; Fig. 1). While during 2016, one was resistant (CSV 19SS), 9 moderately resistant (DSV4, CSV 18R, CSV 19SS, CSV 26R, CSV 216R, CSV 8R, E 36-1, Phule Chitra, Phule Suchitra, and CSV 29R), 8 susceptible (Phule Revati, CSV 14R, Phule Vasudha, CSV 20, Parbhani Moti, RS 585, PVK Kranti and M35-1) and one were highly susceptible (SLR 30). Overall, CSV 19SS performed as the most resistant cultivar, CSV 18R, DSV4, E 36-1 and CSV 8R as moderately resistant and SLR 30, RS 585, M35-1 and Parbhani Moti as susceptible to pokkah boeng over both the years. CSV 19SS was reported as the best performer in previous study (Das et al. 2015), which further supports high pokkah boeng resistance nature of this cultivar. The pokkah boeng rating scale detected around 47% (9 out of 19) of the cultivars (including the susceptible SLR

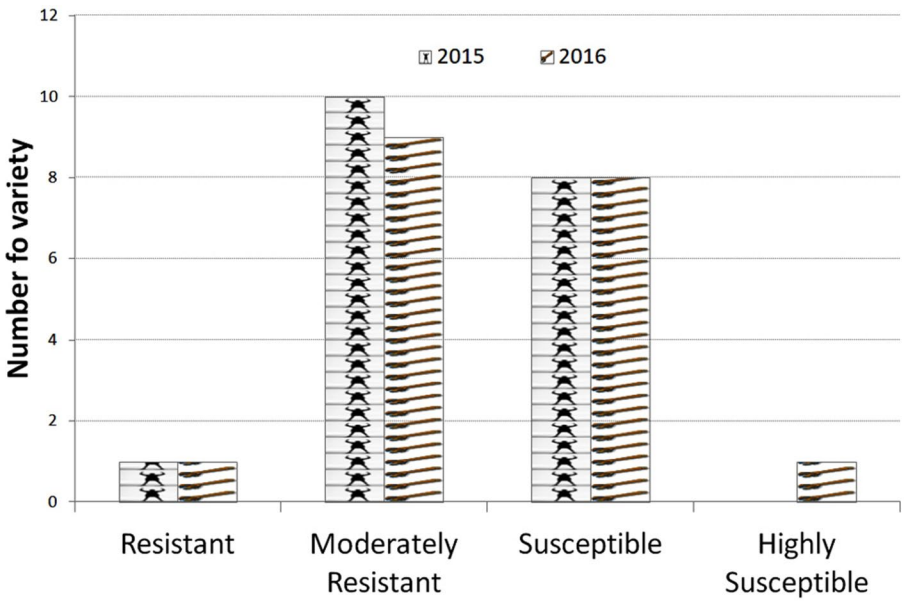

Fig. 1 Pokkah boeng resistance trends in popular rabi sorghum cultivars under artificial inoculation in field conditions

30 and resistant CSV 19SS) with same disease reactions across the years. Considering disease development in aerial plant parts and variable conditions under field in different year, 47% repeatability is quite a good number, which supports the suitability of the scale in deciding pokkah boeng resistance in sorghum. In case of soil borne disease, however, a greater repeatability across environments can be achieved (Das et al. 2018). Testing the cultivars against pokkah boeng under controlled conditions will be helpful in fine tuning their resistance. Each year about 8 cultivars (~42%) were found susceptible (Table 1). This suggests that many of the popular rabi sorghum cultivars carry inherent pokkah boeng susceptibility. This is because it is relatively a new disease and the present day cultivars were released without evaluation against pokkah boeng. The most resistant cultivar CSV 19SS and the most susceptible SLR 30 identified in this study will act as reference line in pokkah boeng resistance and can be utilized for screening of sorghum lines against the disease.

Table 3 Effect of pokkah boeng disease on leaf chlorophyll contents of rabi sorghum cultivars

Cultivar	Reduction in leaf chlorophyll (%)		
	2015	2016	Mean
CSV 8R	14.2 (6.1)[a]	19.2 (11.4)[a]	8.7
CSV 14R	8.1 (2.3)	24.1 (17.8)	10.1
CSV 216R	10.4 (3.3)	23.0 (15.6)	9.4
CSV 18R	23.4 (16.0)	29.2 (24.9)	20.4
CSV19SS	11.6 (4.6)	28.0 (23.0)	13.8
CSV20	14.9 (6.7)	27.0 (21.1)	13.9
CSV 26R	22.5 (14.7)	25.0 (18.4)	16.5
CSV 29R	10.9 (4.1)	21.6 (13.8)	8.9
DSV4	16.4 (8.1)	23.7 (16.5)	12.3
PVK Kranti	15.8 (8.1)	24.1 (17.5)	12.8
Phule Chitra	10.4 (3.6)	30.2 (25.6)	14.6
Phule Revati	19.1 (10.8)	22.9 (15.9)	13.3
Phule Suchitra	6.8 (1.4)	21.4 (13.7)	7.6
Phule Vashudha	24.4 (17.4)	23.2 (16.8)	17.1
Parbhani Moti	12.1 (5.0)	20.4 (13.7)	9.3
M35-1	23.5 (16.0)	33.3 (30.3)	23.2
E 36-1	4.3 (0.7)	14.3 (4.4)	2.6
RS 585	15.9 (7.8)	29.6 (26.6)	17.2
SLR 30	26.5 (20.3)	28.4 (23.6)	21.9
CD at 5%	3.1	4.3	–

[a]Figures in parentheses are original values from which arcsine values are derived

Effects of the disease on leaf chlorophyll

Artificial inoculation of sorghum seedlings with *F. subglutinans* at 30 DAE induced leaf chlorosis on inoculated plants in the field. Reduction of leaf chlorophyll, as measured by SPAD reading ranged from 0.7–20.3% in 2015 to 4.4–30.3% in 2016 among 19 rabi sorghum cultivars. There were wide variations and the reductions were significantly different among cultivars (Table 3). E36-1 registered the lowest reduction in both the years (0.7% and 4.4%). E36-1 is a stay-green genotype with tolerance to terminal drought stress (Haussmann et al. 2002). Its stress tolerant property might have acted against chlorosis development. CSV 29SS, which was resistant to pokkah boeng, however, recorded variable reductions (4.6% in 2015 and 23.0% in 2016) across the years. The susceptible check SLR 30 recorded reduction of 20.3% in 2015 and 23.6% in 2016. SLR 30, RS 585 and M35-1 were among cultivars that showed greatest reduction in leaf chlorophyll (~21%) due to pokkah boeng infection across the years. The results suggest that the disease has relatively more effect on leaf chlorophyll in susceptible cultivars than in the resistant one. As leaf chlorophyll content measured by SPAD chlorophyll meter was reported to have significant effect on plant height, panicle weight and grain yield in rabi sorghum (Talwar et al. 2009), the susceptible cultivars is expected to experience more adverse effect due to pokkah boeng than the resistant one. Correlation coefficients indicating relationship among pokkah boeng incidence, severity and SPAD value have been presented in Table 4. There was strong and significant positive relationship between incidence and severity in both the years ($p=0.000$) (Table 4). SPAD readings did not show significant correlation either with incidence or severity. However, its relationship with incidence was stronger ($p<0.094$) than with severity ($p>0.276$). Stronger relations of SPAD readings with incidence was due to the fact that phase-1 symptoms (chlorosis) contributed directly to incidence but not necessary to severity. Because advancement of phase-1

Table 4 Relationship among incidence, severity and SPAD chlorophyll meter reading in pokkah boeng infected rabi sorghum

Parameter	Incidence (%)		Severity index (%)	
	2015	2016	2015	2016
Severity index (%)	0.834 ($p=0.000$)	0.764 ($p=0.000$)		
Reduction in SPAD chlorophyll meter reading (%)	0.395 ($p=0.094$)	0.410 ($p=0.081$)	0.264 ($p=0.276$)	0.233 ($p=0.337$)

symptom to phase-2 or higher phase that determines severity is highly dependent on environmental conditions in the field.

Present study evaluated 19 rabi sorghum cultivars developed at different regions on India for pokkah boeng resistance using stem injection method of artificial inoculation under field conditions. The cultivars varied in pokkah boeng resistance and CSV 19SS was identified as resistant, CSV 18R, DSV4, E 36-1 and CSV 8R as moderately resistant and SLR 30, RS 585, M35-1 and Parbhani Moti as susceptible. This was the first study of pokkah boeng resistance in popular rabi sorghum cultivars. The information will help selection of cultivar for use in the field. The most resistant and the most susceptible lines identified in this study will act as reference line in pokkah boeng resistance for future studies. The disease showed pronounced effects on development of leaf chlorosis in susceptible cultivar compared to resistant one.

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