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EVALUATION OF HOT WATER TREATMENT ON SEED GERMINATION AND SEEDLING INFECTION OF ARTIFICIALLY INOCULATED COWPEA SEEDS BY *XANTHOMONAS AXONOPODIS* PV. *VIGNICOLA*

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Abstract: Cowpea [*Vigna unguiculata* (L.) Walp.] is an important multipurpose pulse crop subjected to various diseases, among them bacterial blight is one of the important diseases caused by *Xanthomonas axonopodis* pv. *vignicola* (Burkh. 1944) Vauterin *et al.,* 1995. Which is known to cause severe reduction in grain yield as it is both internally and externally seed borne pathogen. The investigation was undertaken at UAS Dharwad to study the effect of hot water treatment on disease and pathogen to mitigate the problem effectively. Hot water treatment by both roll paper towel method and pot culture experiment revealed that 52°C for 10 min., was found effective in eliminating seed borne bacteria *Xanthomonas axonopodis* pv. *vignicola* without affecting seed germination to a greater extent.

Key words: Cowpea; Bacterial Blight; Hot Water; Seed Treatment

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] (2n=22) commonly called as Lobia, is one of the most ancient human food sources and short duration multipurpose pulse crop grown extensively in tropical and subtropical countries. It belongs to family Fabaceae. The name cowpea originated from the fact that the plant was an important source of hay for cows in the south-eastern United States and in other parts of the world (Timko *et al.,* 2007).

Cowpea forms an important component of farming system, being cultivated for seeds (shelled green or dried), pods or leaves that are consumed as green vegetable or for pasture, hay, silage and green manure. The overall grain yields of cowpea in the present traditional systems is low (Singh *et al.*, 1997) due to a complex of biotic and abiotic factors. The abiotic factors that cause yield reduction include poor soil fertility, drought, temperature extremes, excessive moisture, late maturity, acidity and stress due to intercropping with cereals. The biotic factors include insect pests, parasitic flowering plants, as well as viral, fungal, bacterial and nematode diseases.

Among the diseases infecting cowpea, the bacterial disease popularly known as 'bacterial blight' caused by *Xanthomonas axonopodis* pv. *vignicola* (Burkholder,1944) The first symptoms appear on cotyledons of seedlings emerging from infected seed and look reddish and wrinkled. First necrotic lesions are formed on leaves and later the stem is attacked. The pathogen reaches vascular bundles and the disease becomes systemic. It causes yield loss of 2.7 - 92.2 per cent depending on susceptibility of the variety (Kishun, 1989).

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For the successful management of any disease under normal conditions, sanitation, eradiation of primary source and chemical protection at initial stages are some of the measures recommended. However, these measures are not enough, whenever the outbreak of disease occur. Hence, thorough understanding of the disease management strategies and concrete package is necessary to address the menace effectively, so as to save the crop at large. The pathogen is seed-borne and secondary spread is by wind-driven rain, soil, insects and infected plant debris (Anon., 1981; Kaiser and Vakili, 1978). Many researchers identified hot water to be the best component of integrated disease management hence, present investigation with different combinations were studied to standardize the temperature levels and time duration for hot water treatment through roll paper towel method and pot culture experiment.

MATERIAL AND METHODS

Effect of hot water treatment on X. axonopodis pv. vignicola artificially inoculated seeds

The present investigation on bacterial blight of cowpea, caused by *Xanthomonas axonopodis* pv. *vignicola* were carried out during 2011-12 in the Department of Plant Pathology, College of Agriculture, University of Agriculture Sciences, Dharwad. Cowpea seeds of cv., C-152 were artificially inoculated with *X. axonopodis* pv. *vignicola* with a cell suspension of 5 × 10^6 cfu/ml.

Seed inoculation

The bacterial isolates obtained from the infected leaves and seeds were multiplied on YDC agar plates and the cells were harvested in sterilized water.

The concentration of the cell suspension was adjusted to 5×10^6 cfu/ml using spectrophometer at 420nm (OD=0.5) (Spectronic 20 D, Milton and Roy).

Apparently healthy cowpea seeds collected from the field were inoculated with bacterial suspension by keeping the seeds in the bacterial suspension contained in 250 ml side arm flask for one hour. The inoculated seeds were air dried overnight and used for further studies.

Artificially inoculated seeds were treated in hot water separately at 48, 50, 52, 54 and 56°C for 5 and 10 min. in a water bath. The seeds were transferred immediately to a beaker containing ice cold water. The water was drained out and seeds were air dried in shade for 24 hrs and used for further studies.

Seeds were treated at different temperature levels and kept for germination through roll paper towel method and they were also used to plant in pots of 6" size containing sterilized potting mixture of soil, sand and FYM in 3:1:1: ratio. Untreated inoculated seeds used in both the tests served as positive control. Observations for bacterial blight infection were recorded and then per cent disease incidence for seedlings was calculated.

RESULTS

Effect of hot water treatment on seed germination and seedling infection of cowpea

The artificially inoculated cowpea seeds were subjected to hot water treatment at 48, 50, 52, 54 and 56°C for 5 and 10 min. This experiment was conducted in roll paper method and pot culture as explained in Materials and Methods. The data are presented in Table 1, Plate 1.

In roll paper method, the seeds treated with hot water at 52°C for 10 min. were very effective in eliminating the seed borne pathogen as average no. of infected seedlings were 6.33. The germination of seeds was also not much affected (58.00%) as compared to untreated control (72.00%). However, average no. of infected seedlings was minimum in 56°C for 10 min. (1.67) but germination percentage was least (47.00%). In pot culture experiment also, the seeds treated with hot water at 52°C for 10 min was very effective in eliminating the seed borne pathogen as very little infection (3.21 per cent) was noticed in seedlings raised from such seeds without affecting germination (52.00 per cent).

The seeds treated at temperatures of 54 and 56°C though resulted in the effective control of the disease, but these temperature levels had deleterious effect on the germination of seeds than 52°C. The disease incidence and germination of seeds in the untreated control was 23.28 and 76.00 per cent respectively indicating the internally seed borne nature of the pathogen.

DISCUSSION

The seed borne nature of bacterial blight of cowpea was first reported by Shekhawat and Patel (1977). Later Gupta and Chakravarti in 1982 also demonstrated the transmission of *Xanthomonas axonopodis* pv. *vignicola* through the seeds and stated that, the bacterium is both externally and internally seed borne. Hence the present study was conducted to know the better temperature for eliminating the seed borne pathogen without affecting the seed germination. As hot water treatment is eco-friendly, cost effective and removes the pathogen from seed efficiently hence, it can be included in integrated management of seed borne plant pathogenic bacteria.

Table 1: Effect of hot water treatment on seed germination and seedling infection of artificially inoculated cowpea seeds by *Xanthomonas axonopodis* pv. *vignicola*

Treatment		Roll paper towel method		Pot culture experiment		
Temperature (°C)	Time (min)	Germination (%)	Average no. of infected seedlings	Germination (%)	% seedling infection	% leaves infection per plant
48	5	71.00 (57.42)*	25.00	72.00 (58.05)	19.26 (26.03)	7.57 (15. 97)
	10	70.00 (56.79)	21.33	67.00 (54.94)	13.45 (21.52)	6.66 (14.45)
50	5	67.00 (54.94)	16.00	63.00 (52.54)	10.78 (19.17)	5.34 (13.37)
	10	65.00 (53.73)	11.33	60.00 (50.77)	8.13 (16.57)	5.00 (12.92)
52	5	62.00 (51.94)	9.67	55.00 (47.87)	5.62 (13.71)	4.26 (11.91)
	10	58.00 (49.60)	6.33	52.00 (46.15)	3.21 (10.33)	2.12 (8.38)
54	5	52.00 (46.15)	4.67	49.00 (44.43)	1.43 (6.87)	1.00 (5.74)
	10	50.00 (45.00)	3.67	46.00 (42.71)	0.00 (0.00)	0.00 (0.00)
56	5	49.00 (44.43)	2.33	44.00 (41.55)	0.00 (0.00)	0.00 (0.00)
	10	47.00 (43.28)	1.67	40.00 (39.23)	0.00 (0.00)	0.00 (0.00)
Control	-	72.00 (58.05)	31.00	76.00 (60.67)	23.28 (28.85)	10.33 (18.75)
SEm ±		1.01	0.33	1.07	0.36	0.09
CD at 1%		4.01	1.33	4.27	1.42	0.36

***** - Figures in the parenthesis are arcsine transformed values.

The results in the present study indicated that 52°C for 10 min was effective in controlling the bacterial blight of cowpea both in roll paper towel method and pot culture experiment with minimum no. of infected seedlings (6.33) and percentage of seedling infection (3.21%) respectively. Germination was also not much affected at 52°C for 10 min (58.00% and 52.00%) as compared to control (72.00% and 76.00%). Even though 54 and 56°C were effective in eliminating the pathogen but it had deleterious effect on germination of seeds (Plate 11).

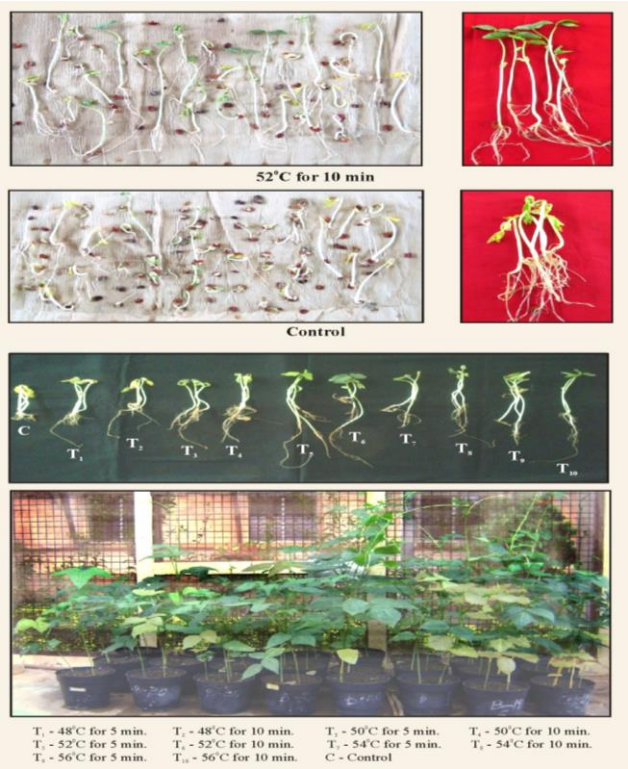

Plate 1: Effect of hot water treatment on seed germination and seedling of artificially inoculated cowpea seeds by *Xanthomonas axonopodis* pv. Vignicola

The present findings are in confirmation with Singh and Verma (1973) who opined that cotton seeds treated with hot water at 56° C for 10 min controlled both external and internal infection of *Xanthomonas malvacearum*. Later in 1999, Sikirou reported that seed dipping in hot water at 60° C for 30 min or 70° C for 10 min or treatment in hot air (65° C for 120 – 144 h or 70° C for 96 h) was eliminating the bacterium from infected cowpea seeds without inhibiting seed germination. However, temperature range depends on the strength of outer coat and water imbibing nature of the seed.

Kolev (1984) also reported that bean (*Phaseolus*) seeds treated at temperature up to 60°C with 45 to 55 per cent relative humidity against bacteriosis (*Xanthomonas campestris* pv. *phaseoli*) effectively controlled the disease. Barua *et al.*, (2007) showed that hot water treatment at 53° C for 15 min was effective in controlling *Xanthomonas campestris* pv. *vignaradiatae and Pseudomonas syringae* pv. *syringae* causing bacterial infections on mungbean, indicating results of the present study to be more and more accurate.

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