Pak J Sci Ind Res 1999 42 (2) 89-92

Biochemical Changes in Wheat Seed due to the Effect of Black Point at Different Levels of Manuring

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(Received 7 December 1995; accepted 22 November 1997)

This study was carried out to demonstrate the effect of black point on the changes of biochemical constituents in wheat seed at different levels of manuring. Starch and total soluble carbohydrates of wheat decreased, while prolamin, glutelin and phenolics increased with the change of manuring schedule levels but method of urea application had no effect on carbohydrate and showed a tendency towards increase in total phenol and prolamin. On the other hand, glutelin and orthohydroxyphenol content showed decreasing tendency under similar conditions. Nitrogen alone, or in combination with cowdung, increased the number of infected wheat seeds; it was also true for methods of N application. Black point pathogens decreased starch, soluble carbohydrates and increased phenolics, prolamin and glutelin content in infected wheat seed. Due to infection marked fluctuation was observed in the contents of reducing sugar, total phenol and prolamin of the seed.

Key words: Biochemical constituents, Wheat seed, Black point, Manure, Fertilizers.

Introduction

Wheat provides potential source of edible protein. Wheat grains contain about 11 to 15% protein, 60 to 70% carbohydrates and small amounts of mineral and other biochemical constituents. Organic and inorganic fertilizers change the biochemical constitutents of seeds. Gluten content was found to increase while starch and total sugar contents decreased with the increasing rates of N fertilizers and their contents were also changed by the methods of N application (Srivastava and Mehrotra 1991).

Black point of wheat is a seed-borne disease caused mainly by *Cladosporium, Curvularia, Drechslera* and *Fusarium* spp. (Adlakha and Joshi 1974; Kulkarni and Hedge 1980). The black point infection was found to range from 8 to 55% (Dey *et al* 1992) and is known to be a cause of reduced seed germination (Fakir 1988).

Plant phenolics, carbohydrates and amino acids have received considerable attention in relation to disease resistance. During host/pathogen interaction, amino acids may act as substrate for the pathogen to synthesise specific proteins related to infection (Titarenko *et al* 1993; Graham *et al* 1990); carbohydrates play significant role for growth and development of pathogen (Jeun and Hwang 1991) and phenolics prevent the disease development (Hahlbrock and Scheel 1989). Carbohydrate content has been reported to decrease while phenolics and amino acid content to increas in *Phytophthora megakarya* infected cocoa pod (Ndoumou *et al* 1996). Protein content in rice seed was changed by *Helminthosporium oryzae* (Vidhyased Karan *et al* 1973).

However, the information about the similar biochemical aspects of wheat seed is scanty. Therefore, the present study was conducted for quantifying such changes in both healthy and black point infected wheat seeds.

Materials and Methods

An experiment was conducted on medium high land of Agronomy Field Laboratory of Bangladesh Agricultural University (BAU), Mymensingh during winter season of 1994-95. The experiment was conducted in a randomized block design with thrice replication, using two varieties (Aghrani = V_1, Kanchan = V_2) in the block and the treatments *viz.* control (220kg urea ha^{-1} in two split (M_1) (Anon 1990), 5t ha^{-1} cowdung +270kg ha^{-1} urea in 3 splits (M_2), 10 t ha^{-1} cowdung + 270kg ha^{-1} urea in 3 splits (M_3), 5t ha^{-1} cowdung + 270 kg ha^{-1} urea in 4 splits (M_4), 10 t ha^{-1} cowdung + 270kg ha^{-1} urea in 4 split (M_5). The land was uniformly fertilized with treated amount of all cowdung, one split of urea, 76.5 kg P_2O_5, 30 kg K_2O and 20 kg S per hectare in the form of triple superphosphate, muriate of potash and gypsum. According to the treatment requirement,

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rest of the splitted urea were applied at 28 DAS (M_1-M_5), 56 DAS (M_2-M_5) and 76 DAS (M_4, M_5) (DAS=days after sowing), respectively. The seeds were sown in lines on November 30, 1994, giving 20 cm line spacing and the plot size was 5m x 2m. The crop was harvested in March 28, 1995. Randomly 200g (about 8000 seeds) seeds were collected from each plot to calculate the percentage of black point infection (number of infected seeds x 100 / total seeds).

Two grams oven dried sample was collected from healthy and black point infected samples. The samples were homogenated in 80% boiling ethyle alcohol @ 8 ml g^{-1}. They were allowed to boil for 8 min and then cooled in running water. The residue was further extracted with 80% ethyl alcohol @ 5 ml g^{-1}. Both these extracts were centrifuged at 2000 rpm for 10 min. The total volume of the filtrate was finally adjusted to 50 ml with ethanol. The extract contained total soluble, reducing and non-reducing sugars, alcohol soluble proteins, phenolics etc. The insoluble parts contained starch, pectins, ash and minerals.

Total sugar and starch was estimated colorimetrically using anthrone reagent (Dubois *et al* 1951). The reducing sugar content was estimated by Nelson's modification of Somogyi's method (Nelson 1944). Total Phenol, orthodihydroxyphenol, prolamin and glutelin were estimated by the methods described by Mahadevan and Sridhar (1982). The data of the experiment were analysed statistically and the mean differences were subjected to Duncan's multiple range test (DMRT) at p=<5%.

Results and Discussion

Biochemical constituents of wheat seed (Table 1) showed that Aghrani contained significantly higher amount of starch and total and reducing sugars than Kanchan. Total phenol and prolamin contents were higher in Kanchan, but orthodihydroxyphenol was more in Aghrani. Glutelin levels showed no significant difference. The biochemical parameters tested were variable with different levels of manuring and methods of urea application (Table 1). The starch content of the varieties showed no significant variation in any of the treatments. Total and reducing sugar content was less when high application of manure was given in 3 splits. When given in 4 splits, it was the highest showing a rise with late application of urea. The total phenol content significantly increased with the higher level of manuring and its content also increased significantly from 2 to 4 split urea application except M_3. Orthodihydroxyphenol content showed a tendency to decrease with the higher amount of manuring and methods of urea application (except M_3). The protein fractions had an increasing tendency with the rate of manuring. Application of urea had marked effect on prolamin synthesis, but glutelin content had a tendency to decrease under similar conditions. It seems that the two varieties synthesized variable amounts of biochemical constituents which may be due to their genetic make up. Carbohydrate content had a tendency to decrease with levels of manuring. Phenolics and protein fractions showed reversible response under the same conditions (except OD-phenol). Reduced wheat starch and soluble carbohydrate due to manuring, was also reported by Singh and Prasad (1974). Improvement in protein and glutelin content and reduction in starch and soluble carbohydrate content due to N fertilizer was observed by Srivastava and Mehrotra (1991) especially when applied in split dosages of N.

Table 1

Biochemical constituents of wheat seed at different levels of manuring and fertilizer application

Variety	Starch (%)	Total sugar (mg g^{-1})	Reducing sugar (mg g^{-1})	Total phenol (mg g^{-1})	OD-phenol (μg g^{-1})	Prolamin (mg g^{-1})	Glutelin (mg g^{-1})
Aghrani	62.1a	10.93a	9.86a	0.56b	90.72a	36.79b	49.13a
Kanchan	55.7b	9.12b	7.89b	0.72a	79.84b	52.48a	45.96ab
Sx	0.61	0.42	0.20	0.014	1.50	2.1	2.07
Manuring							
M_1	55.62	9.13ab	7.69b	0.72b	105.20a	36.37c	42.24b
M_2	56.03	8.99ab	8.15ab	0.70b	108.30a	48.11b	58.80a
M_3	57.33	7.93b	6.50c	0.78a	86.95b	55.40a	58.76a
M_4	56.38	9.51a	7.63b	0.82a	85.96b	59.63a	54.73a
M_5	56.93	9.73a	8.71a	0.82a	83.14b	61.07a	42.95b
Sx	NS	0.45	0.23	0.015	1.67	2.35	2.31

OD = Orthodihydroxy

The number of black point infected seeds was higher in Kanchan and insignificant in Aghrani, but the number of infected seeds significantly increased with the levels of manuring (Fig 1). The highest number of infected seeds was found in M_4 and M_5. The disease incidence gradually increased from two to four split application of urea. It seems that organic manure increased the seed infection which also enhanced with the split application of urea. The important role of nitrogen fertilizer in increasing the incidence of plant diseases has also been reported by many other workers (Sekhar and Prasad 1989; Sharma and Sharma 1991; Dhindsa and Aulakh 1992).

All the biochemical parameters were significantly variable between healthy and infected seeds of both the varieties (Table 2). The infected seed contained lower amount of starch and soluble carbohydrates while phenolics and protein fractions were higher with the same seed as compared to healthy seeds. Black point pathogens reduced 8 to 25% carbohydrates, induced accumulation of 20 to 40% phenolics and 18 to 33% protein fraction over control (Fig 2). Between the two varieties, higher depletion or elevation of constituents was observed in Kanchan than the Aghrani. Among the constituents marked fluctuation were found in reducing sugar, total phenol and prolamin content of infected seeds. It seems that the changes of constituents depend on the inherent character of the variety. The observation that the pathogen increased protein (Kashem *et al* 1992) and phenolics (Dweivedi 1990) while decreased carbohydrates was made by many workers (Sharma *et al* 1992; Vidhyasekaran *et al* 1973; Srivastava and Jaiwal 1991).

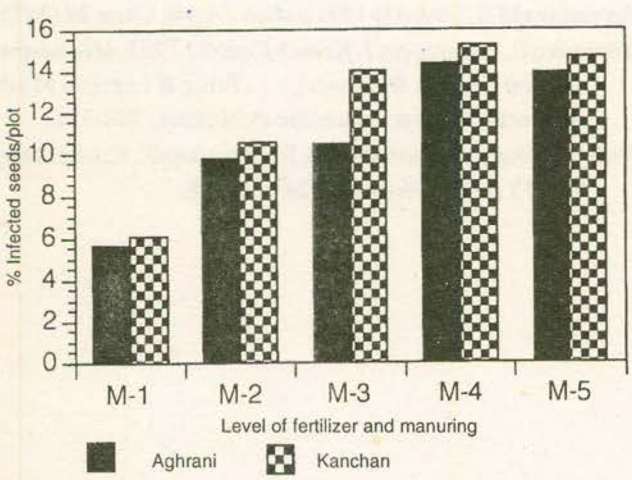

Fig 1. Percent black point infected wheat seeds (number of infected seed/plot) at different levels of manuring and fertilizer application (each value is mean and Sx is 1.88).

The black point disease decreased carbohydrates and increased phenolics, prolamin and glutelin contents of wheat. The reason was that the pathogens used carbohydrates, par-

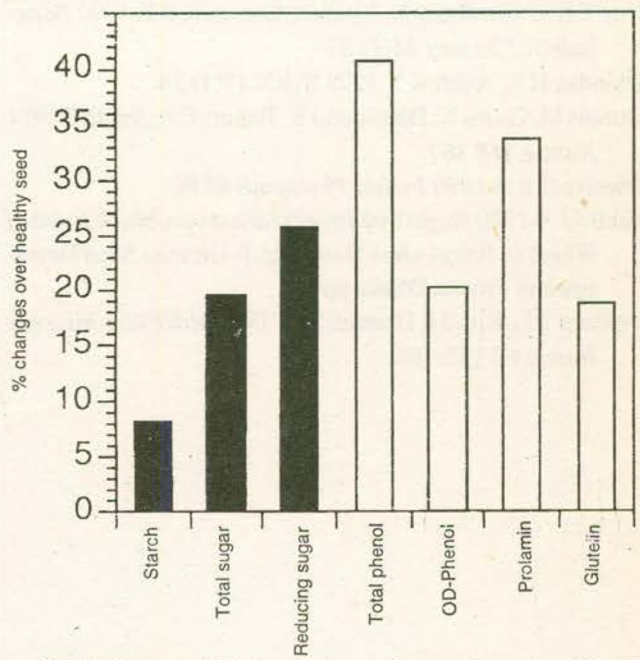

Fig 2. Changes of biochemical constituents due to the effect of black point disease (dark bar, % decreased; white bar, % increased over healthy).

Table 2

Variety wise changes in wheat seed constituents due to black point disease

Variety		Starch (%) (mg g^{-1})	Total sugar (mg g^{-1})	Reducing sugar (mg g^{-1})	Total phenol (µg g^{-1})	OD-phenol	Prolamin (mg g^{-1})	Glutelin (mg g^{-1})
Aghrani	H	62.1a	10.93a	9.86a	0.56c	90.72c	36.79c	49.13bc
	I	55.3b	9.03b	7.65b	0.91a	99.81b	51.74b	53.62ab
Kanchan	H	55.7b	9.12b	7.89b	0.72b	79.84d	52.48b	45.96c
	I	52.9c	7.16c	5.54c	0.88a	105.3a	67.44a	57.27a
Sx		0.61	0.42	0.20	0.01	1.50	2.1	2.07

ticularly reducing sugar and synthesised higher amount of pathogenic protein like prolamin. The blackish color of grain may be due to the complex of pathogenic protein and phenolic compounds or other organic compounds. In fact, the infected plants, try to over come the pathogenic stress by accumulation/synthesis of phenolics and also by synthesis of bioresistant phenolic compounds which prevents the disease development (Hahlbrock and Scheel 1989; Ndoumou *et al* 1996).

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