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Morphological and Isozymic Variations among Karnal Bunt Resistant and Susceptible Genotypes of Wheat – a Comparison

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Two highly resistant genotypes of wheat viz. HD 29 and DWL 5023 and one highly susceptible genotype WL 711 against Karnal bunt (KB) disease were studied for their difference in morphological features, growth parameters and isozyme patterns. It revealed that both the resistant genotypes were bearing higher number of spikelets with short internodes in the spike as compared to the susceptible genotype. In contrast WL 711 had significantly higher number of stomata in sheaths, flag leaf base, booted glumes and rachis. The hair count was significantly high on the glumes and rachis of HD 29 and DWL 5023 than on WL 711. HD 29 possessed significantly narrow glume opening distance between lemma and palea followed by DWL 5023 and WL 711. Moreover, the period between ear emergence and anthesis was short in HD 29 followed by DWL 5023 and WL 711. Out of the twelve isozyme systems performed using seeds and seedlings of the genotypes, majority of them gave rise to comparatively higher number of bands in HD 29 and DWL 5023 than in WL 711. However, specific band(s) for each genotype were very less. Cluster information was the same for morphological data and isozymic banding patterns in Unweighted Pair Group Method with Arithmetic Averages (UPGMA) analysis where both the resistant genotypes together formed a cluster leaving susceptible genotype alone in a separate cluster. Comparison between morphological features and isozyme patterns of the wheat genotypes in relation to KB disease is discussed.

Keywords: Karnal bunt, *Neovossia indica,* wheat genotypes, morphological variations, isozyme patterns.

Karnal bunt (KB) of wheat caused by *Neovossia indica* (Mitra) Mundhur is a potential threat to international trade of commercial grain and wheat germplasm (Royer and Rytter, 1988). The wheat crop is highly prone to this disease at pre-anthesis or spike emergence stage than any other stages of crop growth (Gill et al., 1993). Under natural conditions, maximum infection occurs at this stage while on artificial inoculations disease is more at boot stage (Gill et al., 1993). Moreover, boot leaf is known as the catchment area for allantoid spores of *N. indica* and its sheath is the reservoir and site of infection (Nagarajan, 1991). Therefore, morphological characters of the boot leaf were considered to be of greater significance in relation to resistant or susceptible reaction against KB disease (Nagarajan, 1991). Therefore, morphological characters of the boot leaf were considered to be of greater significance in relation to resistant or susceptible reaction against KB disease (Nagarajan, 1991). Electrophoretic banding patterns of isozymes are frequently predictable since they are dependent on the genetic and nuclear

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condition of the organism (Micales and Bonde, 1995). Hence, isozyme systems have been effectively used as biochemical or genetic markers to screen germplasm of many crops including wheat (Kobrehel and Gautier, 1974; Salinas et al., 1982; William et al., 1993).

Considering these facts, we aimed to know the variations among the KB resistant and susceptible genotypes of wheat based on the morphological features at the boot stage, and isozyme patterns if seeds and seedlings. In addition, we attempted to establish a relationship between the mentioned two parameters of wheat. The resulting relationship is expected to provide avenue to develop morphological and isozyme markers for KB resistance in wheat which would be useful in rapid screening of KB resistant wheat germplasm.

Materials and Methods

Genotypes of wheat

Two bread wheat (*Triticum aestivum* L.) were used. Of these, HD 29 (HD 2160-HD1977/HD 1949-HD 1944/HD 2136) was highly resistant genotype and WL 711 (S 308 x Chris/Kalyansona) was highly susceptible genotype (Gill et al., 1990; Bag et al., 1999). Only one highly resistant durum wheat (*T. durum* Desf.) used was DWL 5023 (CR/LDS//PLC/GARZA) (Singh et al., 1993).

Morphological features and growth characters

Morphological characteristics of the three wheat genotypes recorded are: (a) compactness of spikelets – (i) number of spikelets per spike and (ii) length (in mm) of internodes of spikes, (b) number of stomata (in 1 mm^2 area) on the earhead (sheath), flag leaf area, booted glumes and rachis, and (c) number of hairs (in 1 mm^2 area) on the glumes and rachis. In addition, the role of few growth characters on the infection of resistant and susceptible genotypes of wheat by *N. indica* was studied by taking observations on: (a) the period (in days) between ear emergence and anthesis, (b) glume opening, i.e. the distance (in mm) between lemma and palea.

Isozyme analysis

Seeds or 7-day-old seedlings were used separately for polyacrylamide gel electrophoresis (PAGE) as given by Davis (1964) and Sambrook et al. (1989). Seeds were soaked overnight in water and bits of the seedlings were ground using a mortar and pestle under ice cool condition. Extraction buffer (0.05 M Tris-HCl, pH 7.4) was added @ 1 ml per g of seeds or seedlings. The homogenate was centrifuged in Sigma 3K 30 centrifuge at 10,000 rpm for 20 min at 4 °C. The clear supernatant was retained for immediate electrophoresis. Standard protocols were followed for staining 12 isozymes *(Table 1).* The schematic zymograms of the isozyme bands were prepared based on their relative mobility (Rm) values.

Differences between cultivars were estimated using Pearson correlation coefficients of Genetic Distance (Sokal and Michener, 1958) using morphological characters

Table 1

Resolution of bands and morphism of isozymes in Karnal bunt resistant (R) and susceptible (S) genotypes of wheat

Sl. No.	Enzyme	Protocol	Resolution of bands*	Morphism**	Number of isozyme bands in three wheat genotypes[a]							
					Seeds				Seedlings			
					HD 29 (R)	DWL 5023 (R)	WL 711 (S)	Band(s) with Rm value (s)[b]	HD 29 (R)	DWL 5023 (R)	WL 711 (S)	Band(s) with Rm value (s)[b]
1	Acid phosphatase (ACP)	Shaw and Prasad (1970)	+	M	ND	ND	ND	–	1	1	0	1 (0.71)
2	Alcohol dehydrogenase (ADH)	Tanksley and Orton (1979)	+	P	ND	ND	ND	–	2	3	1	1 (0.43)
3	Alkaline phosphatase (ALP)	Scandalios (1969)	–	–	–	–	–	–	–	–	–	–
4	α-Amylase (α-AMY)	Siepmann and Stegemann (1967)	+	P+	2	2	2	0	2	2	5	1 (0.39)
5	Catalase (CAT)	Glaszmann et al. (1988)	+	P+	3	3	3	1(0.35)	2	2	2	0
6	Esterase (EST)	Shaw and Prasad (1970)	+	P+	11	10	7	3 (0.21, 0.52, 0.55)	9	9	5	2 (0.19, 0.52)
7	Glucose-6-phosphate dehydrogenase (GPD)	Sing and Brewer (1969)	–	–	–	–	–	–	–	–	–	–
8	Isocitrate dehydrogenase (IDH)	Glaszmann et al. (1988)	+	P+	6	5	6	1 (0.56)	5	5	4	0
9	Malate dehydrogenase (MDH)	Shaw and Prasad (1970)	+	P+	12	14	9	3 (0.16, 0.20, 0.30)	5	5	7	1(0.37)
10	Peroxidase (POX)	McDonald and Smith (1972)	+	P	3	3	2	1 (0.42)	5	5	4	1 (0.28)
11	Phosphoglucoisomerase (PGI)	Glaszmann et al. (1988)	+	P+	3	3	4	1 (0.72)	4	4	3	1 (0.78)
12	Shikimate dehydrogenase (SDH)	Glaszmann et al. (1988)	–	–	–	–	–	–	–	–	–	–

* + Band(s) resolved
– Band(s) not resolved

** M Morphism (only 1 band)
P Less polymorphism (7 5 bands
P+ High polymorphism (8 5 bands)

[a] Distribution of bands that depicted in Figure 1

[b] Band(s) present in HD 29 and DWL 711, but absent in WL 711

ND Isozyme analysis not done

and observed isozymic banding patterns. Cluster analysis of matrix distance coefficients were carried out by the unweighted pair group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973).

Results

Morphological variation

Number of spikelets per spike was more in both resistant genotypes than in the susceptible WL 711, but their difference was statistically at par *(Table 2).* The length of internode was significantly high in WL 711 followed by HD 29 and DWL 5023. The time period between ear emergence and anthesis was not significant among the genotypes, although in the case of WL 711 longer time of 11.90 days was required to attain anthesis. In comparison to widest glume opening recorded in the susceptible bread wheat WL 711, the resistant bread wheat HD 29 possessed significantly narrow glume opening followed by DWL5023.

Table 2

Morphological characters of wheat genotypes resistant and susceptible to *Neovossia indica*[*]

Genotypes (with reaction to disease)	Number of spikelets per spike	Length of internode (mm)	Period between ear emergence and anthesis (days)	Glume opening (distance between lemma and palea) (mm)
HD 29 (R)[**]	12.25	4.23	10.95	0.70
DWL 5023 (R)	12.35	3.28	11.20	1.34
WL 711 (S)	11.25	5.10	11.90	1.77
LSD at 5%	NS	0.36	NS	0.28

[*] Data are the mean of 50 spikes
[**] R = Resistant; S = Susceptible

Frequency of stomata present on the floral parts of the three wheat genotypes ranged from 19.46 in the rachis to 62.53 in sheaths *(Table 3).* The array of stomata was found in rows in all types of wheat. Highest number of stomata was observed in WL 711. Among the two resistant genotypes also stomatal number was significantly different in sheath and rachis and it was statistically at par in the flag leaf base and glumes. Number of hair was counted least on the glumes and rachis of WL 711.

Isozyme variation

Out of the 12 enzymes stained, 9 enzymes performed well, of which 6 were moderate to highly polymorphic, 2 were weakly polymorphic and only one was monomorphic *(Table 1 and Fig. 1).* Isozyme activity was not found in ALP, GDP and SDH. In every

Table 3

Frequency of stomata and hairs on the floral parts of wheat genotypes resistant and susceptible to *Neovossia indica*[*]

Genotypes (with reaction to disease)		Number of stomata in 1 mm² area				Number of hairs in 1 mm² area	
		Sheath	Flag leaf base	Booted glumes	Rachis	Glume	Rachis
HD 29	(R)[**]	34.33	39.40	32.13	35.00	223.66	182.46
DWL 5023	(R)	47.60	46.20	25.53	19.46	294.86	182.93
WL 711	(S)	62.53	59.53	61.13	48.93	155.86	121.66
LSD at 5%		4.78	8.56	12.39	8.50	34.24	21.31

[*] Data are the mean of 50 earheads
[**] R = Resistant; S = Susceptible

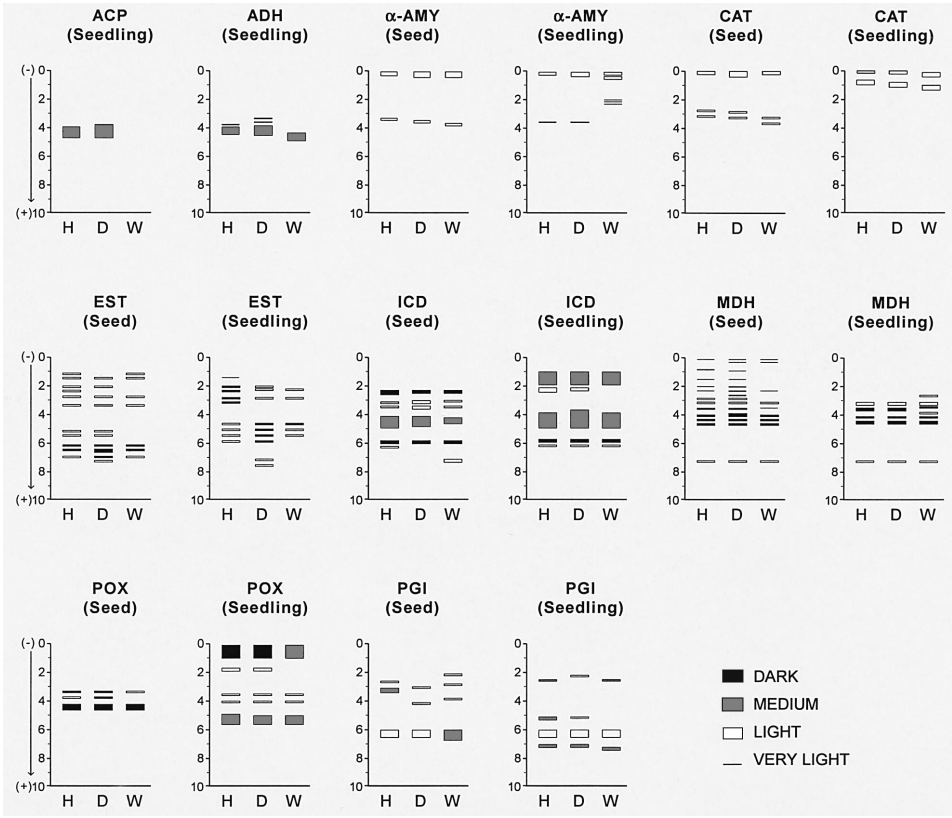


Fig. 1. Electrophoretic phenotypes of three wheat genotypes viz. HD 29 (H), DWL 5023 (D) and WL 711 (W) for 10 enzymes. First two genotypes are resistant and the third genotype is susceptible to *Neovossia indica.* Enzyme abbreviations are spelled in *Table 1*

isozyme system, all the three wheat genotypes expressed more number of common bands. Contrary to this, their specific bands were very less. Majority of the isozymes exhibited higher number of bands in the resistant genotypes HD 29 and DWL 5023 in comparison to the susceptible genotype WL 711. In ACP of seedlings, a fast moving monomorphic band of Rm 0.71 was common between HD 29 and DWL 5023 which lacked in WL 711. In EST and MDH of seeds, a maximum of 3 bands were common in HD 29 and DWL 5023. Their seedlings shared two EST bands of Rm 0.19 and 0.52. Seeds of both resistant genotypes shared only one band of CAT, IDH , POX and PGI isozymes at Rm values 0.35, 0.56, 0.42 and 0.72 respectively. In addition, they shared another band in the case of 5 isozymes, namely ADH, a-AMY, MDH, POX and PGI at Rm values 0.43, 0.39, 0.37, 0.28 and 0.78, respectively besides Rm 0.71 of ACP.

Cluster analysis

Dendrograms developed from the morphological data and isozymic banding pattern, cluster information were found to be the same. In both the dendrograms *(Fig. 2)* two different clusters were observed. The resistant cultivars HD 29 and DWL 5023 are separated in the first cluster while susceptible cultivar WL 711 formed the second cluster.

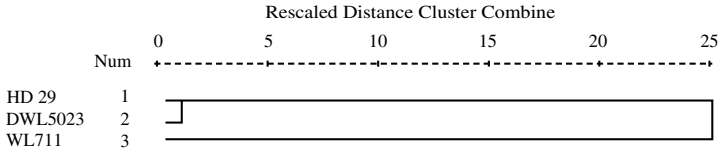
Discussion

Morphological variation does exist among the three wheat genotypes with resistant and susceptible reactions to KB pathogen. But, there was no significant difference in the number of spikelets in resistant and susceptible genotypes, even though both resistant genotypes HD 29 and DWL 5023 were bearing more spikelets in a compact manner in the spikes. The length of internode was minimum in the resistant durum DWL 5023 followed by resistant bread wheat HD 29 and it differed significantly from the susceptible bread wheat WL 711. Gill et al. (1993) also reported minimum internodal distance between the spikelets in durums followed by triticale and bread wheat. In the present investigation, highly compact spikelets in the resistant genotypes might have governed resistance against KB disease as Ahuja et al. (1990) opined that compact arrangement of spikelets in the spikes of wheat varieties is one of the parameters imparting morphological resistance to Karnal bunt. In its contrast, Singh (1992) did not find significant differences between all susceptible and resistant lines in relation to compactness of florets and thought that this character plays very little role. But it is mention worthy that Singh's findings were based on artificial test with syringe inoculation. However, it is quite likely that in nature where sporodia are lifted by wind or rain splash, compact spike may escape infection and provide field resistance particularly in durum wheat.

Period between ear emergence and anthesis was longer in susceptible genotype WL 711 followed by the resistant durum and bread wheat. Singh (1992) also observed that in comparison to resistant lines, the susceptible lines of bread wheat and durum wheats and triticale required more number of days to attain anthesis. It is obvious that the varieties where anthesis takes place early, can overcome infection.

Correlation coefficient matrix

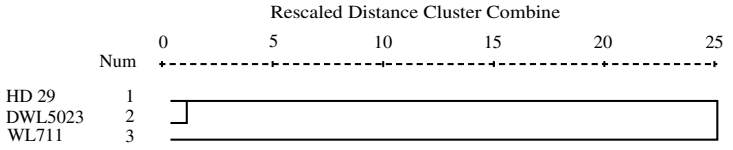
	HD 29	DWL 5023
DWL 5023	**0.9998**	
WL 711	**0.0542**	**0.0001**



a)

Correlation coefficient matrix

	HD 29	DWL 5023
DWL 5023	**0.9833**	
WL 711	**0.9365**	**0.9339**



b)

Fig. 2. Dendrogram using (a) morphological characters and (b) Isozymic banding patterns by UPGMA cluster analysis

Glume opening was significantly more in WL 711 and least in HD 29 followed by DWL 5023. The observation made by Gill et al. (1993) indicated lesser glume opening in resistant durum wheat and triticale. Since in our study, the period of ear emergence in WL 711 was longer and glume opening was more, it may be possible that an enhanced period for infection process was available in susceptible genotype than in resistant one which compels WL 711 to get high infection of Karnal bunt under natural conditions.

The sheath, flag leaf base, booted glumes and rachis of the susceptible genotype possessed more number of stomata. Glumes and rachis of durum wheat had numerous hairs followed by the resistant and susceptible bread wheats. It showed that due to less hairs or in other words smooth surface of the rachis and glumes, there was no barrier to the hyphae of the germinating sporodia to establish infection. Contrarily, densely grown hairs in resistant genotypes restricted the entry of the hyphae that resulted poor infection, which is in agreement with the observation of Singh (1992). Further, Scanning Electron

Microscopy (SEM) of the glume surface of WL 711 and HD 29 revealed that hairs were very few in WL 711 and there was no waxing on the leaves, permitting vigorous growth of *N. indica* on this genotypes (Anonymous, 1996).

In most of the isozyme systems, the number of band was higher in HD 29 and DWL 5023 than in WL 711. Similarly, more peroxidase bands were obtained in powdery mildew and loose smut resistant wheat genotypes (Yang et al., 1984; Arora and Wagle, 1985). With respect to the monomorphic ACP band of Rm 0.71, both resistant genotypes were similar, but they were different from the susceptible genotype.

Banding patterns of the seeds and seedlings of the same genotype were dissimilar. Bosch et al. (1987) also evidenced differences in POX banding patterns resolved from the endosperm and embryo plus scutellum of the same seed of wheat. These observations indicated that use of different parts of the same plant is necessary for assessing isozyme variations. Although HD 29 and DWL 5023 were genetically different genotypes, they expressed similarity by having common bands of the same Rm values. According to Yang et al. (1984) banding pattern is the expression of gene(s). It seems that both resistant genotypes shared some common loci indicating expression of KB resistant gene(s). These results have shown the possibility to develop isozyme markers for KB resistance particularly with the help of sole distinguishing band resolved from the seed and seedlings of only the resistant genotypes.

In conclusion, morphological characters of a plant are still preferred by the breeders in the selection of desirable genotype(s) from a population of germplasm. Recently, Kumar and Nagarajan (1998) emphasized on some plant characters of wheat viz. leaf posture of the flag leaf for KB resistant genotype selection. Compared to this, isozymes, which are genetically controlled, can more accurately project the identity of different species of plants. However, the groupings of the wheat germplasm, as obtained through cluster analysis were exactly similar for both morphological features and isozyme pattern. Therefore, we propose that there must be a correlation between morphological features and isozyme variation of the wheat genotypes and screening of the germplasm for KB resistance may effectively be done either analyzing by any one of these two parameters or by both.

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