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Detection of heat shock protein in bread wheat through ELISA.

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Introduction. High temperature stress is an important abiotic factor that reduces drastically wheat yields in the arid and semi-arid tropics. Howard (1924) reported that for every one degree rise of mean temperature over the range of 12.2–27.53°C, the crop yield is reduced by 4%. To overcome the limits created by higher-temperature stress, a major impetus is on the use of suitable screening techniques to identify heat-tolerant genotypes. Under natural conditions, abiotic stress is usually encountered gradually. Plants, therefore, are exposed to a sublethal stress before being subjected to severe stress. Several studies have shown that plants develop the ability to withstand lethal temperatures upon exposure to sublethal temperatures (known as induction stress). This phenomenon has been termed 'acquired thermo-tolerance' (Hahn and Li 1990). During the induction stress, many stress-inducible genes are triggered ,which alters several physiological and biochemical processes relevant for stress tolerance. Heat shock proteins (HSP) have been known to play a role in cell protection, survival, and recovery in several species (Vierling 1991; Nguyen et al. 1992). Mild heat treatment induces a so-called heat shock response leading to the immediate induction of a set of new proteins or the over-expression of already existing HSPs that persist over time at high temperature. The 90-kDa HSPs are the second most predominantly

expressed HSPs after the 70-kDa family. These proteins appear to impart thermotolerance, because mutant cells with an impaired capacity to make HSP 90 are incapable of growing at higher temperatures (Borkovich et al. 1989).

Materials and Methods. Twelve wheat genotypes/cultivars were used for the present investigation (Table 1). The experiment was conducted on two sowing dates, 18 November and 18 December, 2006. Protein extracted from the leaves of plants from both sowing dates were used separately for experimentation. ELISA tests were developed with minor modifications as described initially by Engvall and Pedlman (1971) and later by Clark and Adams (1977). Here, microtitre plates were coated with different concentrations of proteins in coating buffers keeping the volume constant, i.e., 100 μl/well of soluble antigen. The plates were incubated for 1 hr at room temperature and kept overnight at 4°C. Following a standard washing procedure, the plates were washed with antibody dilution buffer. A 100 μl dilution of primary antibody (anti-HSP 90 sera) was added and the plates were incubated for 2 hrs at room temperature. The plates were washed again with antibody dilution buffer three times and a substrate of 1:1,000 times diluted alkaline phosphate conjugated secondary antibody were added to the plates (rabbit anti-goat I_gG-ALP conjugate) and incubated for 2 hrs at room temperature. After washing the plates three times with dilution buffer, 100-μl substrate solutions were added in each well and incubated for 30 min. The reaction was terminated by adding 100 μl of 1.5 M NaOH solution. The absorbance of the plates was taken 405 nm in an ELISA reader.

Table 1. Twelve wheat genotypes and their pedigrees used in the detection of heat-shock proteins.

Genotype	Pedigree
Acc 8528	not available
DBW 14	Raj 3765 / PBW 343
Halna (K 7903)	HD 1982 / K 816
HD 2808	WH 542 / DL 377-8
HI 385 (Mukta)	HYB 633 / Baza // PR / PKD 25
NP 846	NP 760 . RN
PBN 51	BUL 'S' / FLS 'S'
Raj 3765	HD 2402 / VL 639
Raj 4014	DL 802-5 / K 9011
UP 2425	HD 2320 / UP 2263
WH 147	E 4870 / C 303 // 5339 / PV 18
WH 1003	WEAVER / JACANA

Results and Discussion. In timely-sown conditions, we observed that the OD value at 405 nm ranged between 0.05 (Raj 3765 and HD 2808) to 0.42 (NP 846). In late-sown conditions, the OD value at 405 nm ranged between 0.05 (Halna) to 0.61 (NP 846). The OD values of both days of sowing of different genotypes are presented in Fig. 1.

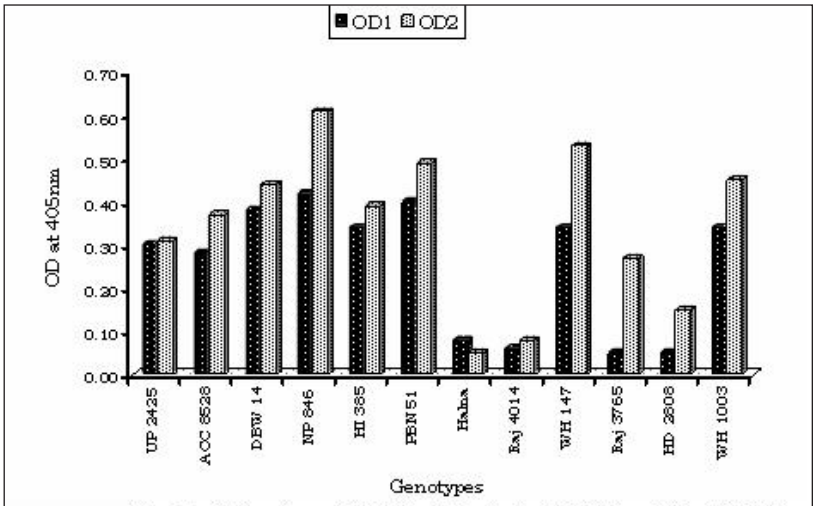

Fig. 1: OD value of ELISA in both D_1 (OD 1) and D_2 (OD 2)

In the ELISA study (which indicates the presence of heat-shock protein), we observed that a majority of the genotypes had high OD values under late-sown conditions compared with the timely sown, and a similar finding was reported by Sharma (2006). Cupina et al. (1979) studied 12 wheat cultivars of varying duration and found that late-maturing types contained more chlorophyll than early cultivars, particularly at heading. This finding indicates that there may be heat-shock protein expressed (HSP 90) in response to heat stress. We found higher ODs for those heat-tolerant genotypes, ACC 8528, DBW 14, NP 846, HI 385, and PBN 51, whereas Raj 4014 had a low OD value and was observed to be heat susceptible. Halna had a very low OD value although it showed heat tolerance on the basis of the heat susceptibility index (result not shown). Because Halna is a heat-tolerant cultivar but matures in a very short period (115 days) in both timely and late-sown conditions, it was not exposed to heat stress, which could be the most plausible explanation of its very low expression of HSP-90 resulting in a very low OD value.

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Performance of brown and black rust resistance genes in some wheat cultivars of central, peninsular and south India.

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Some of the popular wheat cultivars grown in central, peninsular, and southern India were evaluated for seedling and adult-plant resistance to black and brown rusts. Because the source of rust inoculum for Central and Peninsular India lies mainly in Nilgiri Hills in southern India, the cultivars were tested only with Nilgiri pathotypes. Cultivars were raised as single lines in plastic trays (12 x 5 cm) each accommodating 10 lines (8 seedlings/line). Uredosporic dust of individual pathotypes prevailing in the Nilgiri Hills and maintained artificially at the IARI Regional Station, Wellington, was inoculated on the wet surface of the primary leaves of 7-day-old seedlings of the test cultivars by a uniform rub application from base to tip. Inoculated pots were kept in a fine mist created with a manually operated water sprayer making a free film of water on the leaf surface. The plants were kept in a high humidity atmosphere maintained in glass humidity chambers. After 24 hours, the pots were transferred to benches in the glasshouse. Optimum temperature (20°C for brown and 25°C for black rust) and a light regime of 16:8 hours light:dark cycle maintained in the glass houses permitted full expression of brown and black rust pustules after 12 days. Host–pathogen interactions were recorded by following standard international procedures of Johnston and Mains (1932) in brown rust and Stackman and Levine (1922) in black rust. Cultivars also were sown in an open field environment exposing them to natural rust pathotypes prevailing in Wellington to evaluate adult-plant resistance response. Rust intensities were recorded on these cultivars at growth stage 71 (Zadoks et al. 1974) following the Peterson scale (Peterson et al., 1948) for estimating adult-plant resistance.

Seedling and adult-plant response of cultivars are given in Table 1 (p. 81). In the Central zone, seven of eight tested cultivars exhibited seedling resistance to all the pathotypes of brown and black rust prevalent in the Nilgiri Hills. These seven cultivars were HI 8498, HI 8381, HI 1544, HI 1531, HI 8627, DL 788-2, and HD 4672 were free of infection from brown and black rusts at the adult stage; their field resistance is robust only if the inoculum in central India originates from the Nilgiri Hills. Only cultivar HI 1500 of central India showed susceptibility but that was only to one race 77-5 (121R63-1) of brown rust. Fortunately, this genotype has strong adult-plant resistance to brown rust (0 rating). Partial susceptibility (10S) of HI 1500 to black rust is a very positive feature because such incomplete resistance restricts the epiphytotic development of disease so that economic losses do not exceed the threshold (field durability; Parlevliet 1977). The majority of the cultivars of the Central Zone possess gene *Sr2*, which is quite desirable for the purpose of preventing black rust epidemics in this zone. Because of the presence of *Sr2*, the rust resistance seems to be stabile in the Central Zone even after 4–5 decades of utilization of the cultivars possessing this gene. This gene is derived from the cultivar Hope, which is responsible for reducing yield losses to only negligible amounts since the late 1960s in