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Short Communication

Variation in wheat germ agglutinin content under moisture stress

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In spite of its physio-chemical and biological properties being characterized, the physiological role of wheat germ agglutinin (WGA) is still a matter of conjecture [1]. Recently we have reported differential accumulation of WGA in the germinating embroys of different wheat cultivars under osmotic stress conditions [2]. But the effect of water stress on WGA accumulation in the developing grains and its variability in different wheat cultivars under natural conditions is not known. Therefore, to gain insight into the physiological role of WGA in water stress conditions, the variability in WGA accumulation in developing grains of wheat cultivars and its regulation in response to drought has been investigated. These studies have revealed substantial variability in WGA content and its induction by water stress in different wheat cultivars. To determine the molecular basis of this variability we carried out restriction fragment length polymorphism (RFLP) studies using WGA cDNA [3] as a probe.

Plants of different wheat cultivars *viz.*, WL711, PBW299, PBW154, C306 and WH542 were raised in green net house conditions. Individual ear heads were tagged on the day of anthesis defined by the first appearance of bright yellow anthers outside the glumes. The plants were stressed by with-holding water supply at 25 days post anthesis (DPA) till the leaves wilted and drooped completely (5 days after imposition of stress) while the control plants were irrigated regularly. The grains were collected at 30 DPA before the stress was relieved, and individual embryos were isolated. WGA was extracted by grinding the embroys in 50nM HCl (3ml/g fw) followed by extensive dialysis against phosphate buffer saline (pH 7.0). The estimation of WGA was carried out using ELISA with rabbit polyclonal antibodies raised against purified WGA (Boehringer Mannheim) as described earlier. The total soluble protein was estimated by Lowry's method [4].

DNA of leaves was isolated according to the method of Murray and Thompson [5]. The manufacturer digested genomic DNA (5ug) with Hind III restriction enzyme (Bangalore Genei) as per the conditions recommended. Electrophoresis was carried out in 0.8% agarose (Sigma) and the resolved DNA fragments were transferred to nylon membrane (Hybond N, Amersham) by vacuum blotting. Linearised plasmid pNVRI containing WGA cDNA was labeled with DIG labeling kit (Boehringer Mannheim) and used as a probe. Hybridization was carried out under stringent conditions at 65°C in 5% SDS and 0.5M phosphate buffer (pH 7.5). The membrane was stringently washed in 0.2 × SSC, 0.1% SDS at 60°C for 30 min each with two changes. Detection of the bound probe was carried out by using non-radioactive DIG labeling kit (Boehringer Mannheim) as per manufacturer's instruction.

Variability in specific WGA content of embryos of different wheat cultivars was observed (Table 1). The drought-induced increase in WGA content was differential and cultivar dependent. The cultivar WL711 recorded a substantial increase of 174% in specific WGA content of embryos under drought conditions as compared to irrigated control whereas WH542 depicted a decrease of 21% due to water stress. The cultivars PBW299 and PBW154 depicted minimum and maximum values of specific WGA content respectively. These changes suggest that the drought stress specifically regulated the expression of WGA gene in all the cultivars.

Table 1. Effect of drought on specific WGA content (ng WHA ug^{-1} TASP) of the developing grains of different wheat cultivars.

Treatment	Cultivars				
	WL711	PBW299	PBW154	C306	WH542
Irrigated	42±4.7	7.8±0.96	174±21.8	149±13.4	155±16.8
Drought	115±13.0	7.5±0.88	190±24.0	147±12.7	122±11.0

TASP : Total Acid Soluble Protein

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Southern blot analysis of Hind III digested genomic DNA revealed a 10.8 kb band in all the wheat cultivars thus implying absence of polymorphism in WGA gene. Lack of RFLP suggests that the observed variability in WGA accumulation in the wheat cultivars be due to differential regulation of this gene at transcriptional and/or translational level rather than due to differences in genome organization. The absence of RFLP also suggests that WGA is a conserved gene, which further signifies its importance to the wheat plants. Our observations are also contrary to the earlier study [6] reporting a single band of 3 to 4 kb in hexaploid (*Triticum aestivum*) and a diploid (*Triticum lonissimum*) wheat. The appearance of a 10.8kb band in wheat cultivars studied and reported here may be due to insertion or duplication of sequences within the WGA gene.

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