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Cellular Changes and their Relationship to Morphology, Abscisic Acid Accumulation and Yield in Wheat (*Triticum aestivum*) Cultivars Under Water Stress

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Abstract: Water deficit led to a rise in abscisic acid levels and then to the induction of lignin synthesis as a survival mechanism in three different wheat cultivars grown under unfavorable environmental conditions. Photosynthets liberated through reduction of internode length were used in production of multiple layers of mesophyll tissue (thick leaf) and cell wall thickening. Plant growth reduction under drought was caused primarily by reduction in leaf area ratio. Cell sizes were reduced under higher temperature and late sown conditions due to the increased rate of cell division of the cells with different cell wall composition. Drought tolerant cultivar synthesized lignin during rehydration around vascular bundle in order to avoid cavitations. ABA enhanced programmed cell death under unfavorable growth conditions.

Key words: Water stress, cell size, abscisic acid, lignifications, membrane stability

INTRODUCTION

Dehydration is one of the most common environmental stresses for plants and is a bottleneck for agricultural development. There are few physiological processes in plants that are not impaired by water deficit (Barnett and Naylor, 1966; Hsiao, 1973; Lu *et al*., 1989; Ristic and Cass, 1991; Chopra and Kumari, 1995; He and Liu, 1998). Plant responses to water deficit can be analyzed by systematically identifying traits that relate to drought tolerance followed by analysis of the physiological, cellular and biochemical basis of the trait. The responses need to classified based on adaptations that allow plant cells to continue growth and development and those that allow plant survival and those that generate a response to lethal disruption of function.

Abscisic acid has numerous effects on plant growth and reproduction that are expected to reduce plant productivity and vary between shoot and roots (Saab *et al*., 1990), as well as between watered or water stressed conditions (Sharp, 2002). The effect also depend on the amount of ABA applied (Chen *et al*., 2003), growth stage (Sharp, 2002), temperature (Dodd and Davies, 1994) and dynamics of drought imposition (Bahrun *et al*., 2002). ABA accumulation has long been assumed to constitute an adaptive factor under water stress. ABA is well known as a stress hormone and plays an important role in the plant response to water stress at both a whole-plant (Zhang and Davies, 1990; Davies and Zhang, 1991) and cellular level (Pla *et al*., 1993; Straub *et al*., 1994). The functional role of ABA in the adaptation of a variety of plants to water stress has been well documented and reviewed (Zeevaart and Creelman, 1988; Davies and Jones, 1991; Mansfield and McAinsh, 1995). Genetic variation influencing endogenous ABA content under water stress might be amenable to selection for improved drought resistance (Read *et al*., 1991; Quarrie, 1993).

Three cultivars of *Triticum aestivum* (hexaploid wheat) were sown at different dates: C306 (drought tolerant and sown in rain fed conditions), HD2285 (Temperature tolerant and late sown) and HD2428 (drought sensitive and normal sown). To investigate the cause of drought tolerance in C306 when compared with HD2428 and HD2285, endogenous ABA accumulation and its role in anatomical and morphological changes were analyzed. The aim of the present study was to assess the effects of ABA on morphology under water stress and consequently on the yield of the crop.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Wheat cultivars C 306 (water stress tolerant), HD 2285 (heat tolerant during grain filling period) and HD 2428 (water stress susceptible) were planted under normal (15 November, 2003) and late-sown (15 December, 2003 and 15 January, 2004) conditions. Experiment was repeated to record various parameters. Sowing was done in 30×30 cm earthen pots filled with sandy loam soil and farmyard manure in 3:1 under a natural environment. Each pot was fertilized with 120 kg ha^{-1} of N, 90 kg ha^{-1} of P and 60 kg ha^{-1} of K. Four seedlings were maintained in each pot. Plants were watered, as required and stress was imposed by a lack of water supply for 4 days. Plant protection measures were taken as needed. The water stress treatment was implemented at ear emergence (1 cm length) and at grain filling stage (half grain). A seven days period was allowed for the revival of the plants from water stress. The flag leaf of the mother shoot was selected for all anatomical analyses and to estimate the Relative Water Content (RWC), endogenous abscisic acid (ABA), heat shock protein and calcium. Each value of RWC is presented as a mean of three replicates. For yield analysis five ears were selected from the mother shoot at the time of harvest. Maximum, minimum and mean temperature was higher while relative humidity decreased at the time of development of flag leaves in January sown plants (Table 1).

Statistical Analysis

The yield components were assessed by using a complete factorial randomized design. Standard error was calculated for RWC and ABA data and is included in the graphs. The critical deference was calculated with a statistical package for agriculture (opstate).

Water Relations

Leaf relative water content was calculated as:

$$RWC = \left(\frac{FW\text{-}DW}{TW\text{-}DW}\right) \times 100$$

where, FW is the fresh weight in the pot, DW is dry weight, after drying samples in an oven at 80°C to constant weight and TW is turgid weight, after saturation in distill water of flag leaf for 6 h.

Table 1: Weather data during crop sowing and flag leaf development in different months

Month	T max (mean)	Tmin (mean)	ΔT (mean)	T mean	RH (M)	RH (E)	RH (mean)
November	27.4	10.6	16.8	21.1	80.4	32.3	56.4
December	19.9	9.3	10.6	21.1	91.0	60.4	75.7
January	17.4	7.3	10.1	12.3	94.5	63.2	78.7
February	24.0	9.3	14.7	16.6	87.0	40.6	64.4
March	37.5	21.4	16.1	29.5	64.0	29.6	46.8
April	Plants harvested in mid April						

Analysis of Endogenous ABA

After determination of the fresh weight, leaves (ten leaves) were immediately frozen and stored at -20°C until ABA analysis. After grinding in liquid nitrogen with a mortar and pestle, the leaf samples were extracted overnight at -20°C in 80% Methanol (10 mL per g FW). The extract was filtered twice with Whatman No. 1 and evaporated in vacuo; then the sample was redissolved in 2 mL of phosphate buffer (pH 8.0). The results are expressed as μg ABA per g DW of flag leaf. The ABA contents were determined using high-performance liquid chromatography (Waters, USA) with photodiode array (PDA) detection at 254 nm. Analytical grade acetic acid (Excel AR grade) and methanol (Gradient grade) were obtained from Merck (India) in 2003. The abscisic acid (Extra pure) used for standard was purchased from Sisco Research Laboratory (India) in 2003.

Light and Electron Microscope Measurements

All samples for microscopy, were taken from fully developed flag leaves around 11.00 A.M. Flag leaf sections for ultrastructural characterization were fixed overnight at 4°C in 2% (v/v) para formaldehyde- 2% (v/v) glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.2), post fixed in 2% (w/v) osmium tetra oxide (OsO4), embedded in a mixture of Epon and Araldite after a standard dehydration procedure. Semi-thin leaf cross-sections were used for light microscopy or tissue identification prior to Transmission Electron Microscopy (TEM). Light microscopy sections were stained with 1% (w/v) Toluidine Blue O in 1% (w/v) sodium borate. Ultra thin sections (silver/gold) for TEM were collected on copper grids with a support film of formvar and carbon, which were glow discharged to promote section recovery and adhesion. The sections were counterstained in 2% (w/v) uranyl acetate for 15 min followed by lead citrate solution for 3 min and then examined with a Philips CM-10 electron microscope (Netherlands) at 60 KV.

Lipid Extraction and Separation

Lipids were extracted from the flag leaf tissue with chloroform: methanol (2:1 v/v) solution and silica gel 60 F254 20×20 cm TLC plates with a 20×2.5 cm concentrating zone (Merck-Germany) were used for separation. Lipid elution was carried out using a solvent system of chloroform: methanol: acetic acid: water (50: 25: 8: 4). Spots were located by spraying with 20% ninhydrin prepared in 80 % acetone in water and heating at 120°C for 1 h (Lepage, 1966).

Extraction and Separation of Heat Shock Protein

Leaf tissue (0.5 g fresh weight) was ground with liquid N_2 to a fine powder. Protein was extracted in 2 mL of Tris-Hcl buffer, 0.1 mL of N, N, N, N-Tetra methyl ethylene diamine, pH (7.5). Samples then were centrifuged at 12000 g at 4°C for 20 min and supernatant was collected. The protein concentration was determined by Bradford (1976) method. The protein samples were precipitated by adding 10 mL of 10% (v/v) trichloro acetic acid. The precipitate was collected by centrifugation at 16000 g for 15 min at 4°C, washed with acetone and dried. The pellet then was dissolved in 2 mL of SDS-PAGE sample buffer (65 mM Tris-HCl, 10% glycerol, 2% SDS, pH 6.8, 5% b-mercaptoethanol) (Laemmli, 1970). Proteins were separated by discontinuous SDS-PAGE with an electrophoresis unit (Genei)) using a 4% stacking gel and 12.5% running gel. Gels were stained over night with colloidal Coomassie blue G-250.

RESULTS

Relative Water Contents and Endogenous Concentration of ABA in Flag Leaves of Wheats

The relative water content (RWC) of wheat cultivars flag leaves under irrigated and water stress conditions (Fig. 1A-C) decreased with age. There was significant reduction in RWC under water stress in all cultivars. Genotype C306 retained a significantly higher RWC when subjected to water stress conditions for all sowing dates. In contrast, HD2285 exhibited an intermediate value, while HD2428 showed the lowest value under water stress. This declining trend in RWC was similar for the second and third sowings at the ear emergence and grain filling stages, respectively. HD2428 which had the longest flag leaves was better able to regain water content in comparison to C306 and HD2285 grown under similar conditions. There was more water loss from flag leaves developed at high temperature of all cultivars sown in January.

Genotypic differences were evident from the recovery of RWC for flag leaves with different areas and capacities for ABA accumulation (Fig. 2A-C). The endogenous ABA level was higher in C306 than in HD2428 and HD2285. In general, the ABA content increased with the loss of RWC and later was dependent on the surface area available for transpiration and vital root system for uptake of water. The plant roots were shallow and suberized and the root tips were thicker under the unfavorable environment of late-sown conditions (January). ABA accumulation was greater at grain filling stages under water stress, as the plants were approaching senescence. Moreover, the ABA level was increased in the flag leaf of all cultivars under altered environmental conditions for crop growth. January-sown plants had

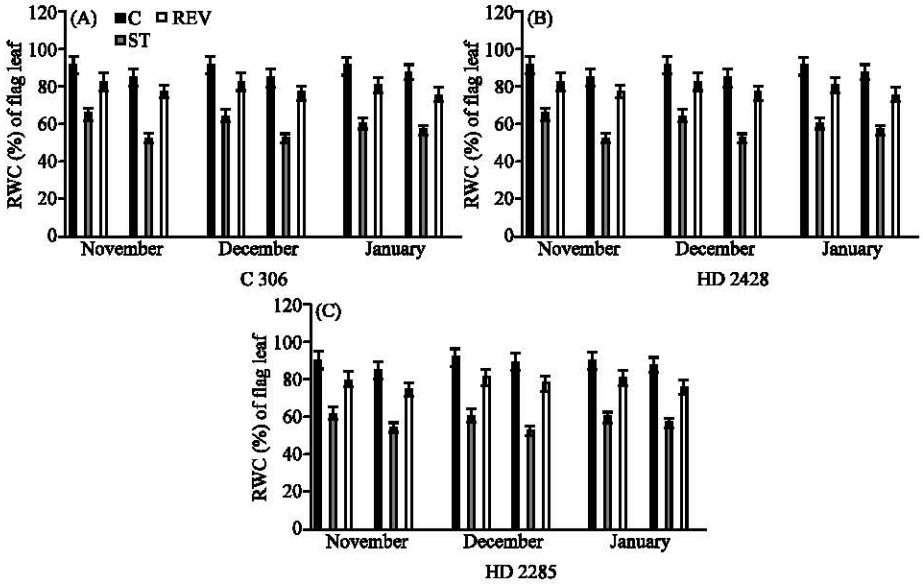

Fig. 1: (A) Relative water contents (%) of flag leaf in C306 at the ear emergence and grain filling stages under water stress at different dates of sowing; (B) Relative water contents (%) of flag leaf in HD2428 at the ear emergence and grain filling stages under water stress at different dates of sowing and (C) Relative water contents (%) of flag leaf in HD2285 at the ear emergence and grain filling stages under water stress at different dates of sowing

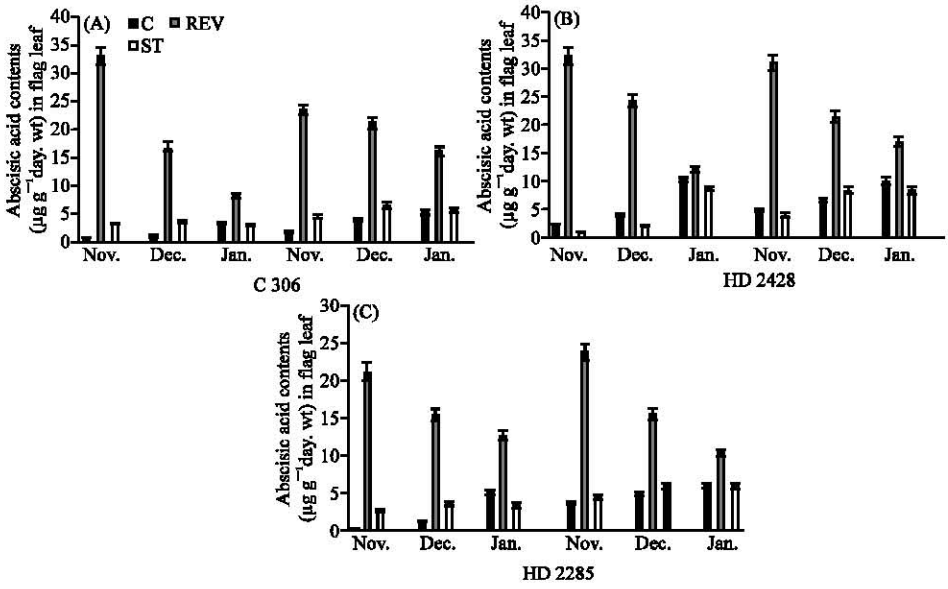

Fig. 2: (A) Abscisic acid contents (µg g^{-1}day wt. of flag leaf) in C306 at the ear emergence and grain filling stages under water stress at different dates of sowing. C-Control, ST-Stress, REV- Revival; (B) Abscisic acid contents (µg g^{-1} day wt. of flag leaf) in HD2428 at the ear emergence and grain filling stages under water stress at different dates of sowing. C-Control, ST-Stress, REV- Revival and (C) Abscisic acid contents (µg g^{-1} day wt. of flag leaf) in HD2285 at the ear emergence and grain filling stages under water stress at different dates of sowing. C-Control, ST-Stress, REV- Revival

lower levels of ABA at the grain filling stage than at the ear-emergence stage with similar values of RWC. However, in January-sown plants, the temperature-tolerant cultivar had lowest ABA concentration. Furthermore, the ABA concentration was dependent on maturity of different cultivars and later was controlled by the number of tillers produced by the cultivars. Anthesis and harvesting of the crop for C306 was 10 days later when compared with HD2428 and HD2285.

Cellular Changes and their Relationship with Morphology in Flag Leaves of Wheats

The electron micrographs (Fig. 3A-C) showed genotypic differences in the shapes and sizes of the chloroplasts and organization of the thylakoid membranes within the chloroplasts (B). These electron micrographs revealed the vacuolation of thylakoid membrane with aging in HD2428. The grana and stroma thylakoid organization in the chloroplasts and cell metabolism were altered to various extents when the cultivars were planted in January (A). The starch accumulation and amyloplast size were also reduced. Degradative processes were increased (i.e., liposome formation, indistinct stroma and grana thylakoids, accumulation of hydrogen peroxide in apoplastic region) in ABA containing plants grown under favorable conditions. The folded cell wall in all cultivars indicated involvement of cell wall-related expansin proteins. The chloroplasts of these plants developed peripheral reticulum (C) and mitochondria and chloroplast cell organelles were abnormal in development. Grana thylakoids were poorly developed, starch granules vanished and chloroplasts showed vacuolation with amorphous materials. Furthermore, plastoglobuli were absent in these chloroplasts.

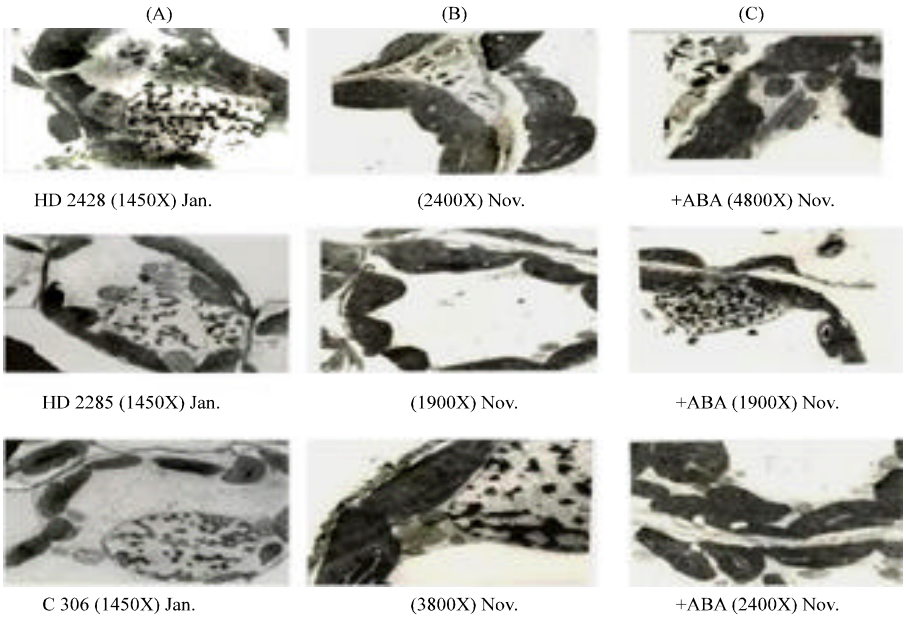

Fig. 3: Electron micrographs illustrate the morphology of chloroplasts in HD2428, HD2285 and C306 in January-sown plants (A), in control, i.e., November sowed plant (B) and seed from (A) planted in November in addition to normal seeds (C). Chloroplasts were compared at the ear emergence stage in fully expanded flag leaves. Arrows highlight plastoglobuli (dense spots) and starch grains (sg). Sampling involved three plants (flag leaf per plant) from all cultivars. Electron micrographs highlight anomalies in mesophyll cell ultrastructures in different cultivars: Degradative processes e.g. liposome formation, indistinct stroma and grana thylakoids, accumulation of hydrogen peroxide in apoplastic region and folded cell wall are increased in ABA-containing plants grown under favorable conditions, indicating the involvement of cell wall-related properties and especially expansin proteins. The chloroplasts of these plants developed peripheral reticulum. Arrows define the position of the plastoglobuli (B) and amorphous material (C). The locations of the chloroplast (ch), cell wall (cw), grana (g), peroxisomes (p) autophagy (Aph) and elongated mitochondria (m), degenerating palisade parenchyma (pp), condensed chromatin in nucleus (n) in each image are denoted by the respective symbol. A mitochondrion with an amorphous inclusion (arrow) and one-containing poorly developed cristae are shown

The stroma thylakoids were damaged in the chloroplasts of stressed plants and the number of plastoglobulii increased (Fig. 4A, B) in comparison with normal plants following the genetic programme of senescence at ear emergence and the grain filling stage. The appearance of vesicles between chloroplast and nucleus was a characteristic feature of cells in HD2428 and HD2285 during rehydration. Liposome originated from the chloroplasts, bound to the nuclear membrane and caused the fragmentation of the nucleus in susceptible cultivars at the senescent stage (Fig. 4C). Peroxisomes and glyoxysomes appeared in cells under stress conditions and during the grain filling stage in all cultivars. Clatherin-coated vesicles could be seen in mesophyll cells of the thermotolerant cultivar. Occasionally,

Fig. 4: Continued

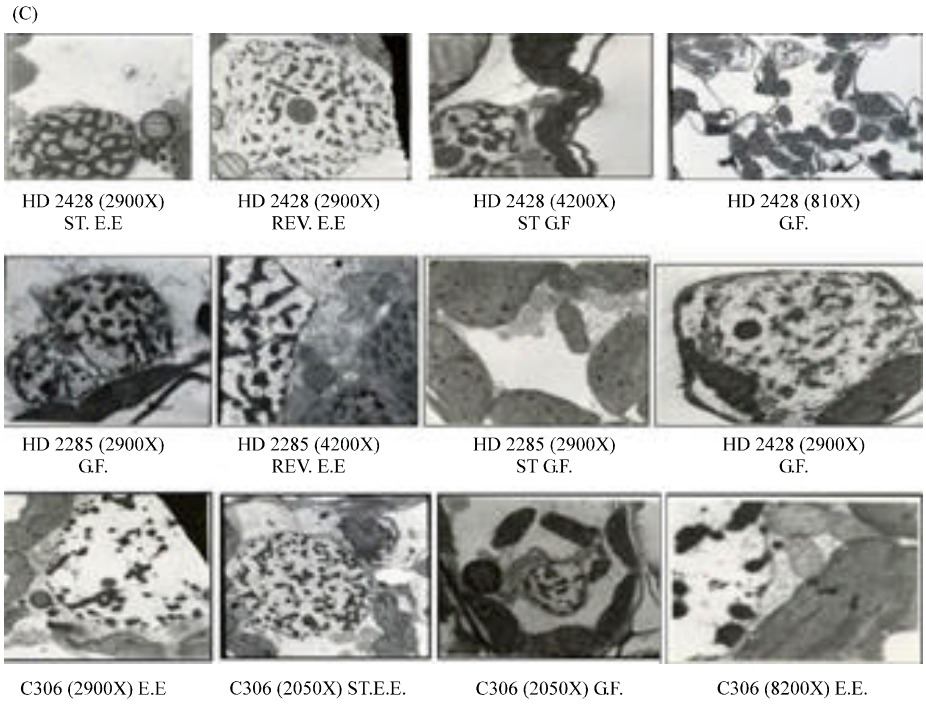

Fig. 4: (A) The originated liposomes and the number of plastoglobulii in chloroplasts increased tremendously under water stress. The degeneration of cellular organelles increased. (B) Electron micrograph showing morphological characteristics in flag leaves harvested from the temperature-tolerant cultivar HD2285. Arrows highlight plastoglobulii (Pg), Nucleus (N), glyoxysome (gl), peroxisome (P), cell wall (CW) and chloroplast (ch). The number of plastoglobulii and peroxisomes increased under water stress conditions. Liposomes originated after damage of chloroplast membranes and interact with nucleus and (C) Electron micrographs illustrate the morphology of chloroplasts in HD 2428, HD2285 and C306 and changes in mesophyll cells undergoing natural senescence (PCD) and water stress-induced changes at ear emergence (E. E.) and grain filling (G. F.) stages. Arrows highlight plastoglobuli (Pg-dense spots) and starch grains (sg), Liposomes (Lp), peroxisomes (p), glyoxysomes (gl), condensed nucleus (n), nucleolous (nu). The chromatin material was condensed in the nucleus and the number of plastoglobuli, liposomes and glyoxysomes increased; mitochondria are confused with peroxisomes. The liposome continued to increase in size and interacts with the nucleus. Sampling involved three plants (flag leaf per plant) from all cultivars

multivesicular bodies and vacuolar autophagy of mesophyll chloroplasts were observed, always with absorbance by the central vacuole. Autophagy of the chloroplast was often characterized by the disintegration of external membranes and disorganization of the grana (Fig. 5A). An apoptotic xylem vessel was observed with degenerating mitochondria and nucleolus. Mechanosensitive plasma membrane channels opposed by the cell wall marked the involvement of calcium in cell signalling during rehydration. The presence of the endoplasmic reticulum, ribosomes and Golgi apparatus indicated *de-novo* synthesis and

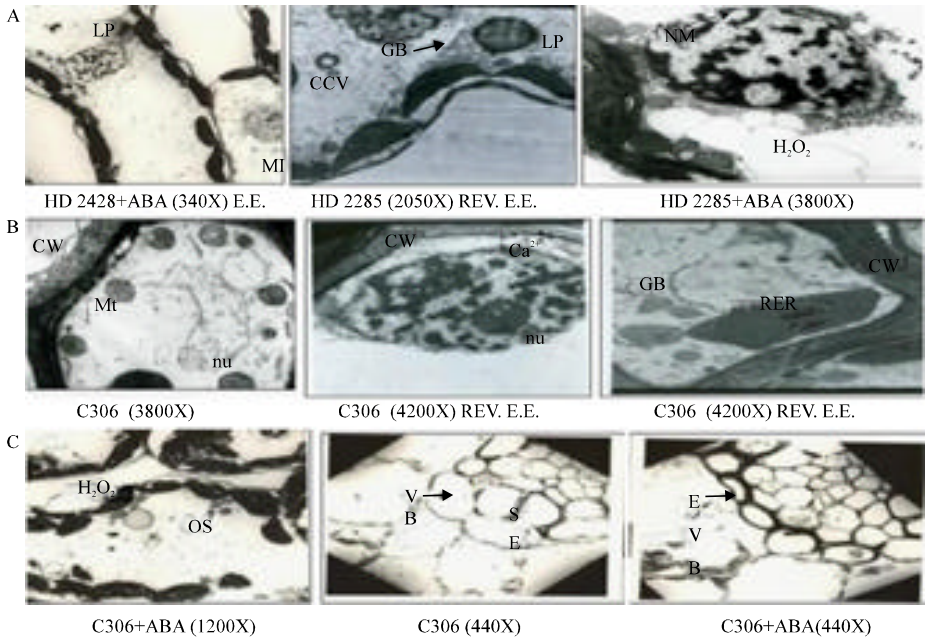

Fig. 5: Electron micrograph showing morphological characteristics and abnormalities identified in flag leaves harvested from different cultivars. Arrows highlight multilamellar body (MLB), liposome with nucleic acid inclusion (LP), clatherin coated vesicles (CCV), broken nuclear membrane (NM), appearance of nucleolus (nu), hydrogen peroxide (H_2O_2) accumulation in cytoplasm, appearance of glass formation in the cytoplasm and nucleus of mesophyll cell of HD2285 (A), in xylem cell undergoing apoptosis; mechanosensitive calcium channels in plasma membrane opposed to cell wall (Ca^{2+}), golgi bodies and rough endoplasmic reticulum appear in vascular parenchyma cell in response to water stress (B) and appearance of the oleosomes (OS), vascular bundle (VB), sieve element (SE) and lignified vascular bundle (LVB) in C306 (C)

modification of proteins in C306 vascular bundles in response to water stress (Fig. 5B). The accumulation of hydrogen peroxide, formation of oleosomes and lignification of vascular bundles were observed in the drought tolerant cultivar in the reverse cytogenesis study (Fig. 5C). Heat shock protein (43 KD) was observed in all cultivars, whereas the total protein content was reduced in the roots and leaves of ABA-containing seeds grown in favorable conditions as compared to November-sown control plants (Fig. 6A). Shoot growth was stimulated at this temperature and with a normal supply of water when compared to plants grown in January. Root proliferation was inhibited in the horizontal direction and the total root mass was reduced as compared to control plants (Fig. 6B). The inhibitory effect was displayed in all tillers. The cell walls were folded in cells of all cultivars in order to restore their normal metabolism and size under favourable growth conditions. In addition, the flag leaf area was reduced with a delay in sowing (Fig. 7A). Partitioning of photosynthets was shifted towards the roots with temperature changes at different sowing dates. Tiller emergence was evident in the axil of the first leaf of plants grown in December (Fig. 7B). However, the plastochrons and phyllochrons number was not changed.

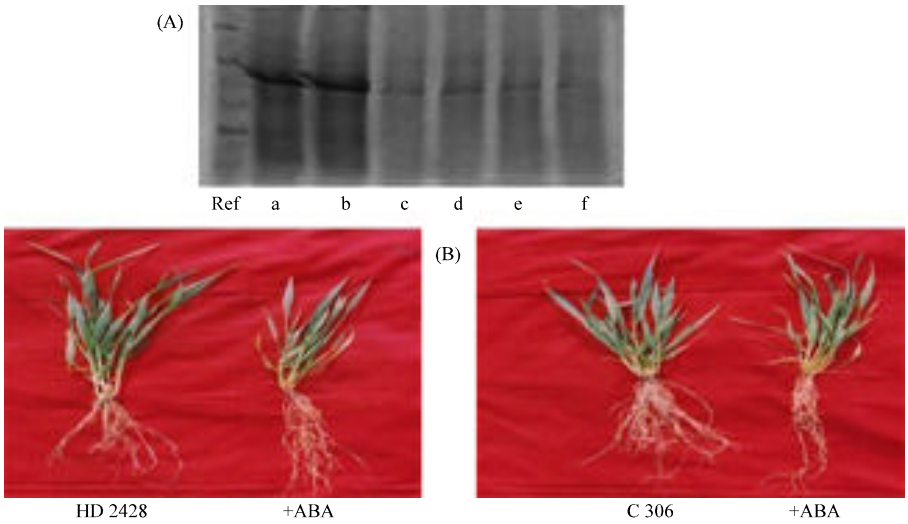

Fig. 6: (A) Heat shock protein (molecular weight 43 KD) was constitutively expressed under November-sown conditions. Standard marker (Ref), (a) flag leaf of HD 2428, (b) HD2428 +ABA, (c) root tissue of HD2428, (d) HD2428 +ABA, (e) root tissue of C306, (f) C306 +ABA. (B) Shoot and Root growth of both cultivars of wheat were reduced by endogenous abscisic acid in seeds of HD2428 and C306; the root length was increased in vertical direction

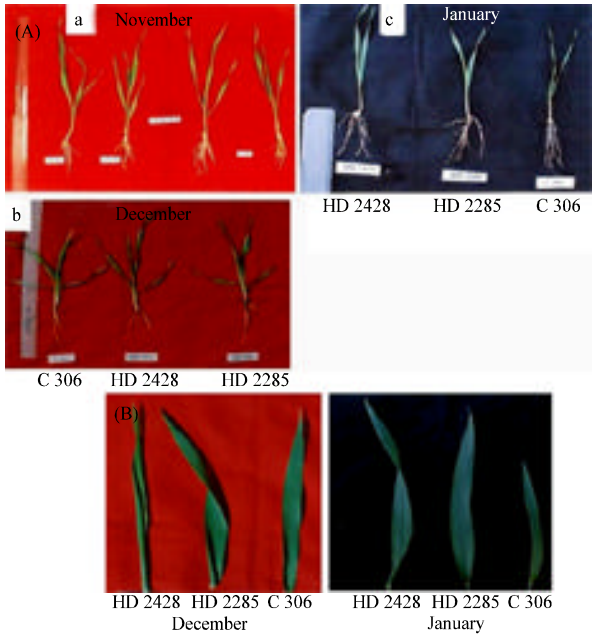

Fig. 7: (A) Dry matter partitioning in wheat seedlings from different sowing dates (a) November, (b) December and (c) January. HD2428-normal, HD2285-Thermotolerant, C306-Drought tolerant. (B) Flag leaf size in different wheat cultivars grown in December and January

The cell volume was reduced due to water loss when the control plants were subjected to water stress (Fig. 8). Furthermore, the cell volume was reduced in late-sown cultivars due to the lignification of cell wall (Fig. 9). In C306, the vascular bundle was lignified during revival from water stress. Larger bulliform cells might be beneficial for slowing water loss and maintaining turgidity in drought-tolerant cultivars (Fig. 10). Guard cells in C306 retained water under stress conditions (Fig. 11).

No qualitative changes in the phospholipids composition of chloroplasts were observed in drought-tolerant, thermo-tolerant or susceptible cultivars when planted in November and December. A sharp decrease in the total number of lipids was evident in plants sown in January (Fig. 12). The phospholipids; Phosphatidyl Glycerol (PG), Phosphatidyl Choline (PC),

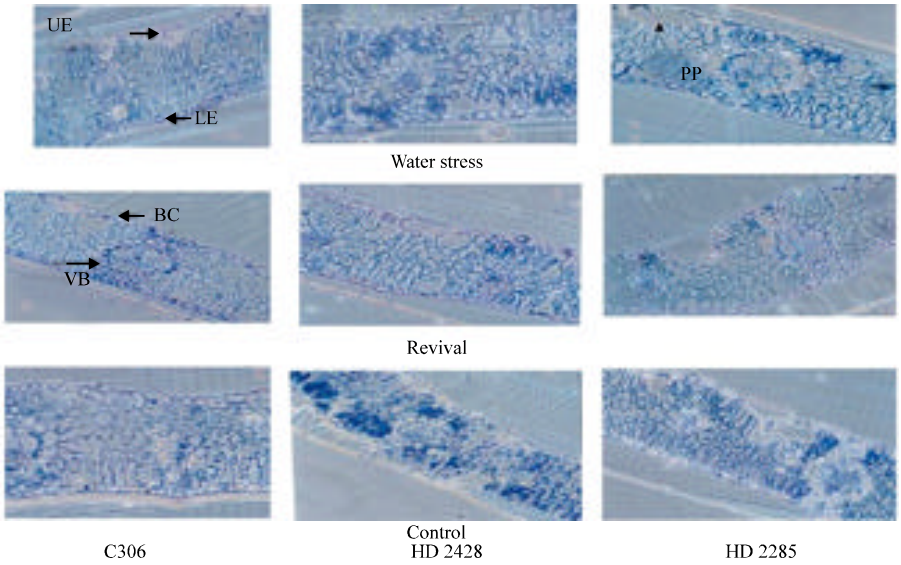

Fig. 8: Transverse sections of flag leaves of three wheat cultivars under light microscope (20X); lignification of vascular bundle in C306 after revival of water stress; upper and lower epidermis (UE and LE), palisade parenchyma (PP), bulliform cell (BC) and vascular bundle (VB)

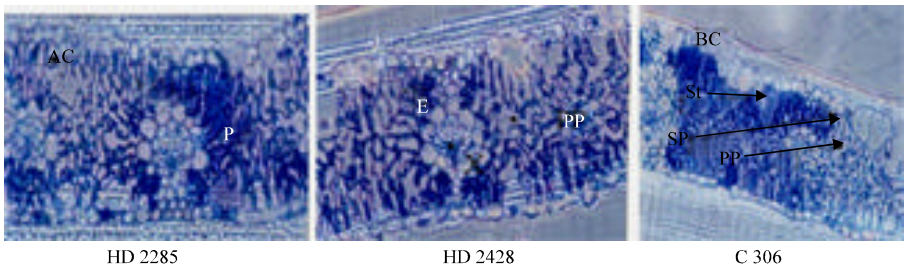

Fig. 9: Transverse sections of flag leaves of three cultivars sown in January under light microscope (20X) displayed reduced cell size and lignification of all cells; epidermal cells (E); xylem (X); bulliform cells (B) ; vascular bundle (VB) and phloem (P); palisade parenchyma (PP); spongy parenchyma (SP); stoma (St)

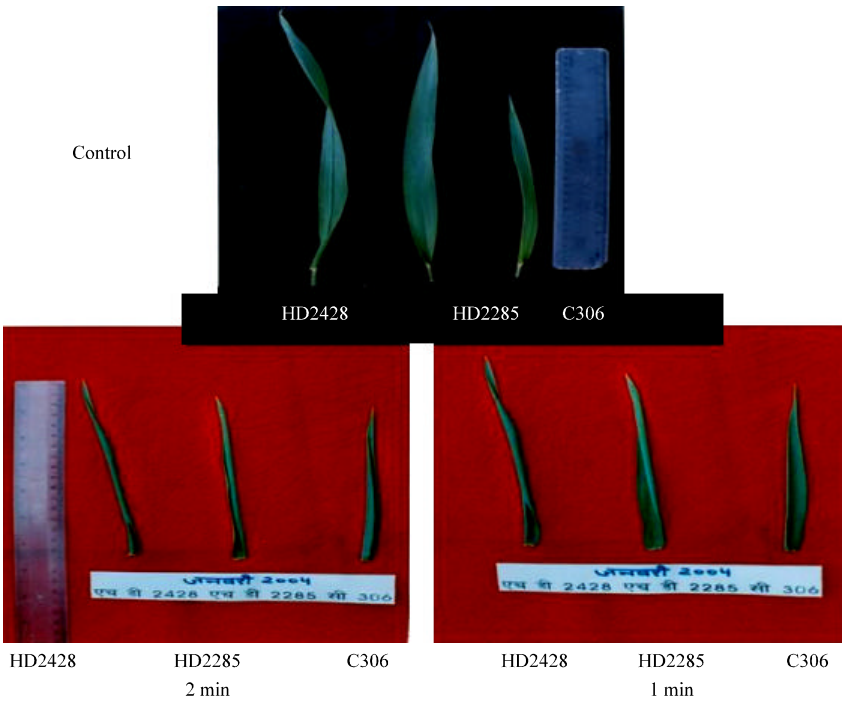

Fig. 10: Water loss from detached flag leaves of HD2428, HD2285 and C306 sown in January at 11:00 a.m. after 1 and 2 min

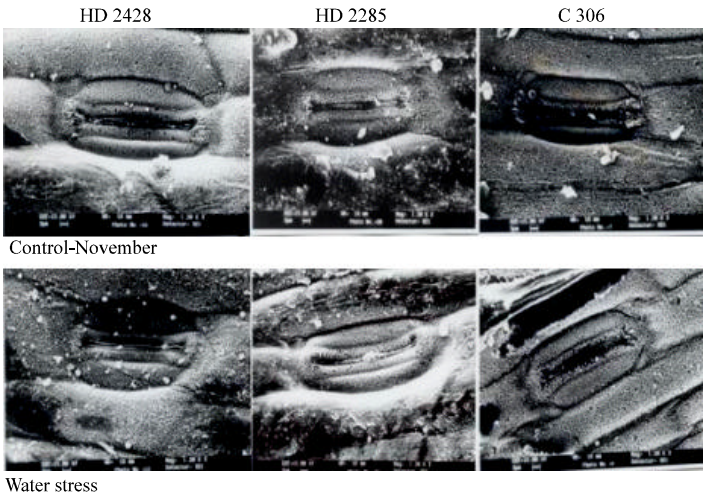

Fig. 11: Morphology of closed stomata of HD2428, HD2285 and C306 wheat cultivars under normal water supply and water stress

Phosphatidyl Ethanolamine (PE) and Phosphatidyl Inositol (PI) are important constituents of chloroplasts, while monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) are exclusively present in chloroplasts. Cardiolipin (PC) the main constituent of mitochondria was the only lipid detected under January-sown conditions where flag leaf

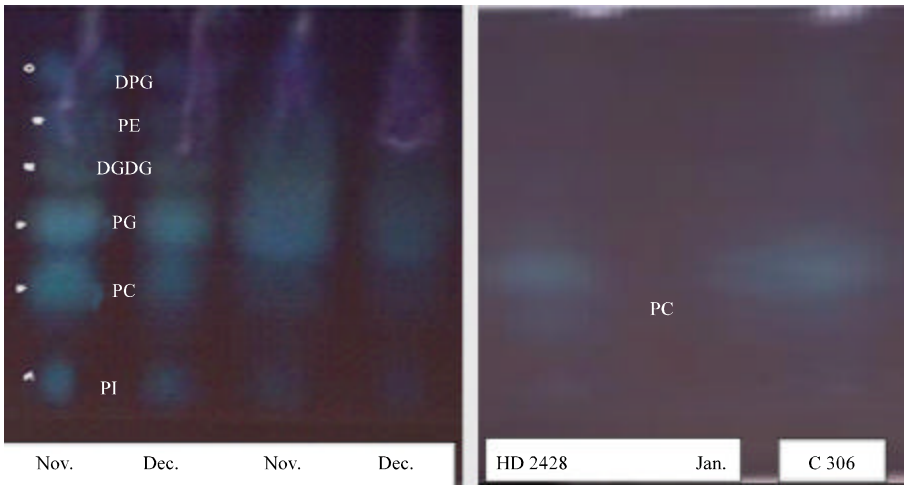

Fig. 12: Six ninhydrin-positive lipids were detected in drought-sensitive and tolerant cultivars under November- and December-sown conditions; intensity of the spot was lower in the latter case. Cardiolipin, the main constituent of mitochondria, was the only lipid detected under January-sown conditions when the flag leaf developed under high temperatures in both cultivars

Table 2: Calcium concentration in flag leaf of drought-sensitive and -tolerant wheat cultivars under late-sown (water stress) conditions (Values are mean of three replicates)

Cultivars	Date of sowing	Concentration of calcium (%)
HD 2428	Nov	0.12
	Dec	0.06
	Jan	0.19
C 306	Nov	0.16
	Dec	0.07
	Jan	0.23
CD at 5%	Variety	0.0011
	Date of sowing	0.0014
	Variety X date of sowing	0.0020

developed under high temperature. The reduced oxidative damage in these cultivars demonstrated the higher stability of the organelle membranes under low-temperature conditions. The calcium content was also higher with a delayed sowing and in the C306 cultivar (Table 2).

The grain number, weight and 100 seed weight were decreased with delay in sowing (Table 3, Fig. 13). Yield losses were higher when water stress was created at the ear emergence stage. Normally sown cultivars took 82 days for ear emergence while subsequently sown plants took 42 and 24 days for ear emergence. The duration for anthesis was reduced from 14 days to 7 days. Therefore, crop duration was reduced and crop matured in the order of HD2428 > HD2285 > C306. The wheat seedlings were allowed to senescence and the lateral roots grew out of existing roots; thus, these roots were inhibited in ABA-containing seedlings from January grown plants (Fig. 14). We have additional evidence that these cultivars were symplasmic loader (Fig. 15) but can adjust osmotically under drought conditions.

Table 3: Seed Weight (g) of five ears from mother shoot and 100 seed weight (g) (Values are mean of three replicates) of C306, HD2285 and HD2428 under water stress at the ear emergence and grain-filling stages

		Control		Stress at ear emergence		Stress at grain filling	
Cultivar	Date of sowing	Seed weight (g)	100 seed weight (g)	Seed weight (g)	100 seed weight (g)	Seed weight (g)	100 seed weight (g)
HD 2428	November	15.6792	4.9552	14.5254	4.9322	13.2730	3.8190
	December	11.0362	4.0938	10.0816	4.5942	9.7472	4.0490
	January	8.6953	3..8460	6.7100	3.9320	5.8930	3.4820
C 306	November	13.4728	4.3786	14.4258	4.8200	14.4326	5.0978
	December	12.5128	3.5584	15.0500	4.6850	6.8010	3.2224
	January	10.0744	4.4574	9.7790	4.1990	9.3360	3.4300
HD 2285	November	15.3856	4.4274	13.6192	4.1010	10.7304	4.0510
	December	13.7444	4.6942	11.2228	4.8290	9.2580	4.0580
	January	9.0330	4.2230	8.2630	4.3150	7.8942	3.8995
CD at 5% stress		0.4900	0.1830				
Variety		0.4900	N.S.				
Date of Sowing		0.4900	N.S.				
Stress × Variety		0.8490	0.3170				
Stress × Date of sowing		0.8490	0.3170				
Variety × Date of Sowing		0.8490	0.3170				
Stress × Variety × Date of sowing		1.4710	0.5490				

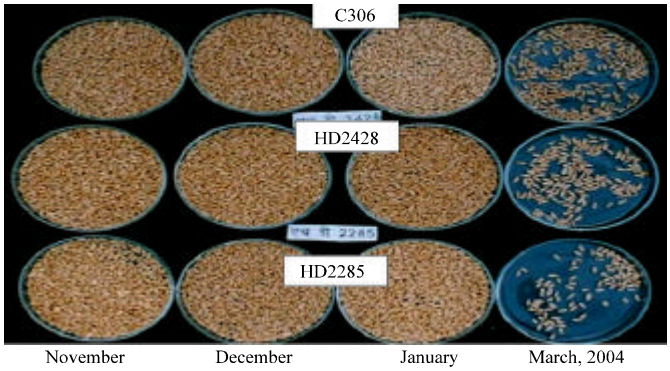

Fig. 13: Numbers and sizes of seeds per plant of C306, HD2428 and HD2285 cultivars sown at different dates

Fig. 14: New root growth (lateral) in HD 2428 drought-susceptible and C 306 drought-tolerant wheat cultivar during senescence of seedlings of normal seeds and ABA-containing seeds in Petri dish at 25°C in the laboratory

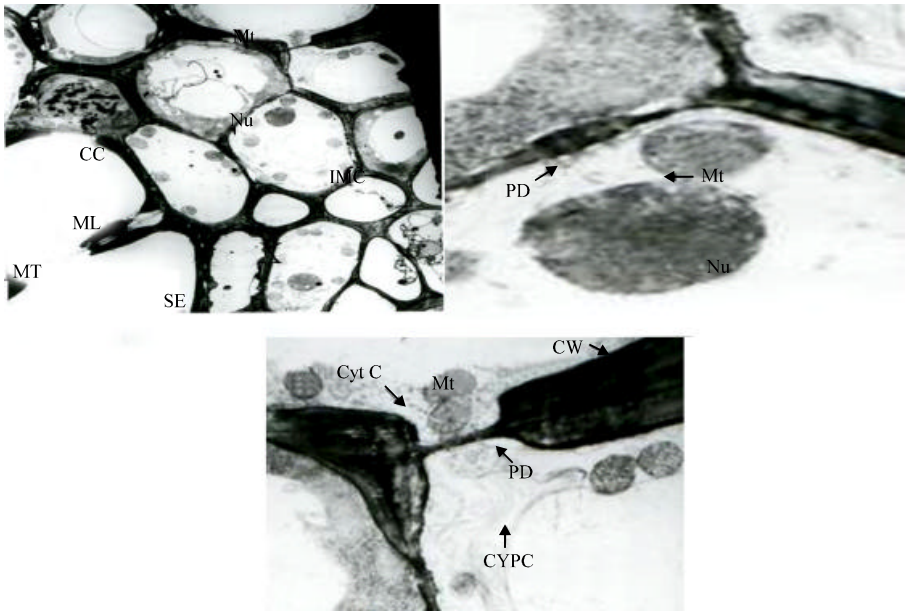

Fig. 15: Electron micrograph show morphological characteristics of plasmodesmata identified in flag leaves harvested from drought-tolerant cultivar, C306. ABA enhances Apoptosis of xylem vessels and lignifies vascular tissue under drought conditions. Micrograph depicts companion cell (CC), mitochondria (Mt), intermediary cell complex (IMC), functional plasmodesmata (PD), nucleolous (nu), xylem vessel (X), sieve element (SE), microtubule (MT), cytochrome c (Cyt c), cell wall (cw), cytoplasmic cyclosis movement (CYPC), middle lamella (ML)

DISCUSSION

Our plant cell results suggest that the initiation of water deficit-induced ABA accumulation was in fact brought about by weight loss of the leaf tissues as reflected by changes of cellular volume, rather than by water parameters (Jia *et al*., 2001). As previously Taylor *et al*. (2005) discussed the highest ABA contents in C 306 might be related to the damage in the photosynthetic tissue of young tillers under water stress and to the availability of xanthophyll precursors for ABA. Cytosol ABA increases during water stress as a result of synthesis in the leaf, redistribution within mesophyll cells, import from the roots and recirculation from other leaves. This in turn gives early warning for stomatal closure to save water through hormone redistribution in the plant.

Thylakoid disruption accompanied by the appearance of plastoglobuli was the most universal structural response of the inner membrane system of cells under stress and correlates well with the rate of photosynthesis suppression. A previous study reported that parts of the long peripheral stroma thylakoid of wheat plants lie in concentric circles under water stress (Freeman and Duysen, 1975), but they lack the typical spatial orientation along the longitudinal axis. This study also showed that the structural contact between adjacent chloroplasts was a result of the deformed stromal zones. The transformational changes provoked by the water deficit were likely of an adaptive nature with regard to the organelle's function. A decrease in the number of phospholipids accompanied by peroxisome

proliferation indicated their role in providing the carbon skeleton for synthesis of secondary metabolites in flag leaves under higher temperature conditions. Modifications in the lipid composition, as well as changes in the fatty acid unsaturation, could have dramatic consequences for the physical properties of cell membrane. The reduction in size or cross-sectional area of chloroplasts in January-sown plants could be accounted for by the reduced contents of C18:3 and C16:3 fatty acids in membrane lipids and a decrease of similar magnitude in the amount of chloroplast lamellar membranes (McCourt *et al*., 1987). In chloroplasts, lipids are involved in the organization of fine structures and also in maintaining the spatial orientation of pigments, quinones and proteins necessary for photosynthetic function. Cha-Um *et al*. (2007) had reported significant degradation of chlorophyll a, total chlorophyll and total carotenoid in the leaf tissues by high glucose and ABA application and their positive correlation to maximum quantum yield of PSII, quantum efficiency of photosystem and non-photochemical quenching, respectively. The low efficiency of light capture in photosystem ii caused low growth, in term of root length, plant height and dry matter.

The cellular changes reported here in January- and November-sown plants were consistent with Programmed Cell Death (PCD) events. The cellular processes were hypothesized to be strongly dependent upon cellular levels of reactive oxygen species (Apel and Hirt, 2004). The PCD markers include condensation of chromatin; autophagy, mitochondrial alterations, chloroplast invagination and thylakoid disorganization were in concurrence with these studies (Ishikawa, 1996; Selga *et al*., 2003; Olmos *et al*., 2006). The chloroplast peripheral reticulum is implicated in the rapid transport of metabolites into or out of the chloroplast because of the large increase in surface area of the chloroplast inner membrane that it provides. Moreover, the association of the chloroplast peripheral reticulum with the inclusion of the mitochondria supports the hypothesis of enhanced inter-organelle transport and communication as cells progress through the PCD pathway. The peripheral reticulum observed in our study had been proposed to be involved in calcium modulation of PCD events affecting membrane permeability and metabolite transport across the chloroplast envelope (Kratsch and Wise, 2000). The number and size of plastoglobuli increased substantially in chloroplasts following exposure to drought (Munné-Bosch *et al*., 2001) supports our findings. Plastoglobuli are proposed to function in the storage of thylakoid components such as lipids, plastohydroquinone and tocopherol (Murphy, 2001) in the synthesis and recycling of lipophilic products arising from oxidative metabolism during stress and the metabolism of molecules derived from carotenoid cleavage (Ytterberg *et al*., 2006).

Typically as water content of the plant decreases, cells shrink and cell walls relax. This decrease in cell volume results in lower turgor pressure and the subsequent increase in concentration of solutes in the cell. The plasma membrane becomes thicker and more compressed because it covers a smaller area than before. Turgor reduction is the earliest significant biophysical effect of water stress and turgor-dependent activities such as cell expansion, leaf expansion and root elongation are the most sensitive to water deficit.

The ABA responses reflect adverse effects on source activity, i.e., current photosynthesis, accelerated senescence and a decrease sink ability to attract and utilize available photosynthets, under both water stress and changed environmental conditions (high temperature). As lateral roots are formed by dedifferentiation and proliferation of mature pericycle cells, the decreased formation of secondary roots under late-sown conditions might be due to precocious recruitment of pericycle cells for secondary vascular growth, or alterations in auxin distribution or signaling (Baima *et al*., 1995). In wheat, ABA

reduced the formation of secondary roots even at low temperature conditions and inhibited growth in the horizontal direction under normal planting.

Many of the symplasmic loaders translocate oligosaccharides of the raffinose family (Holtaus and Schmitz, 1991; Beebe and Turgeon, 1992; Haritatos and Turgeon, 1996) and can build up a high osmotic potential in the intermediary cells, so that water will enter and raise their turgor. We have demonstrated that C306 is a drought-tolerant genotype because it synthesized lignin using reactive oxygen species-induced Cu-Zn-SOD antioxidants in the vascular bundle during recovery from water stress. These antioxidants are proposed to function with lignification in the apoplast and to protect the cell against fatal mutations caused by O_2 molecules in the nucleus. The cultivars can use smaller vessels on recovery and resist cavitations (breakage of the water column) by lignification of vascular bundle under water stress conditions. However, ABA reduced the size of the smaller vessels due to secondary growth; thereby reducing the efficiency of water flow in wheat at low temperature and high temperatures. Furthermore, lignin is water-insoluble and thus immobile; therefore, lignin must be made where it is found. In fact, lignin is initially formed in the middle lamella and primary cell wall (PCW) of cells such as xylem vessel elements and phloem fibers (Wardrop, 1971). The initial site of lignifications provides a clear example of a wall-localized enzymatic activity *in vivo* (Abdulrazzak *et al*., 2006). Our anatomical studies revealed that ABA enhanced apoptosis in vascular bundles at low and high temperatures. Therefore, senescence was enhanced under higher temperatures for growth and development. In reverse cytogenesis studies, ABA results in the formation of glassy matrix (Bruni and Leopard, 1992; Buitink and Leprince, 2004) in HD2285 and C 306 cells under favorable temperature conditions. An efficient repair and full reconstitution of membrane integrity during rehydration would be a prerequisite to cell survival as revealed in the mesophyll cells of HD2285 and vascular bundles of C306.

Cell sizes were reduced because of the increased rate of growth, rapid division of smaller-sized cells and different cell wall compositions (indicated by vascular bundles and epidermal cells) under higher temperatures. Secondary metabolism, such as in the synthesis of lignin, utilizes more energy. Therefore, photosynthets liberated by internode reduction are used for production of thick leaves (multiple layers of mesophyll tissue) and cell walls. Tissue hydraulic conductance may have limited cell expansion and leaf growth. Inhibition of cell expansion results in a slowing of leaf expansion early in the development of water deficits. A drop in leaf water potential is coupled with a decline in leaf turgor potential unless the species is capable of osmotic adjustment (Boyer and McPherson, 1975; Begg and Turner, 1976; Turner and Jones, 1980). Water stress can also affect leaf area by accelerating leaf senescence. For example, such stress reduced the period for grain growth and enhanced plant maturity. In addition, a shorter duration for the ear emergence and grain development in all cultivars further reduced the grain yield. The final outcomes were reduced internode length, plant height, leaf area and duration of developmental phases in wheat.

Reduced cell expansion as a primary response to water deficit serves to reduce plant water use but also leads to diminished plant productivity. If the reduction of total plant water use is not sufficient to sustain turgor, then transpiration is further reduced by stomatal closure. Wilting is an expression of turgor loss and manifests in various ways, such as leaf rolling, depending on the plant species. The C306 flag leaves were turgid and their yield was higher in comparison to HD2428 and HD2285 under drought conditions. Reduced cell expansion also has primary effects on meristematic development of yield components in wheat. The reduced spikes size or the tiller initials leading to potentially reduced yield is an irreversible structural effect that is difficult to amend by rewatering. The meristematic tissues

are generally positioned within the plant in a relatively protected environment as compared with that of a fully expanded leaf. Either a sever water stress or ABA transported from stress-affected organs can arrest meristem development even at relatively high meristem water levels, as was revealed by the reduced size of spikes in all three cultivars with delayed sowings. It is evident from our data that ABA had an inhibitory effect on grain growth. The decreased 100 seed weight of wheat could have been due to reduced endosperm cell expansion arising from water stress that limited the maximal storage capacity of the kernels at the ear emergence stage. Moreover, the flag leaf and spike sizes were reduced in plant relieved from moisture stress at ear emergence. A reserve deposition process might be affected by a water deficit at the grain filling stage. Narrow vessels formation under the influence of ABA and early disconnection of grain could be additional reasons for the smaller grain size under water stress, particularly in late-sown crops. Our study with fresh tissue further showed that the grain yield was diminished not only due to reduced leaf area and spike size but also due to the infertility of spikes and inability of the pollen tube to reach the egg cell or ovule due to the thickness of ovary wall in lignified tissue (observation under microscope).

In wheat, growth, differentiation and protein synthesis in the immature apex were concomitantly reduced by water stress of -12 bars (Barlow *et al*., 1977). Water stress changed the pattern of protein synthesis (i.e., lignin synthesis occurred and later was dependent on osmotic adjustment) in C 306. The abscisic acid concentration and plant response were temperature-dependent in wheat flag leaves displaying water deficiency (Machàcková *et al*., 1998). The hormone did not alter the pattern of heat shock protein synthesis (43 KD, HSP). The sensitivity of cell expansion to osmotic stress has stimulated studies of various genes that encode proteins involved in the structural composition and integrity of cell walls. Genes encoding for enzymes such as S-adenosylmethionine synthase and peroxidases, which may be involved in lignin biosynthesis, have been shown to be controlled by stress.

When the apex differentiates into a spikelet in wheat, flag leaf initiation has already begun. A stress period at this stage and increase in ABA concentration reduced the size of flag leaf relatively more in drought sensitive cultivar. If a metabolic adaptation leads to the restoration of the flag leaf size or spikelet number, the genotype would be stable. But once the final spikelet number or leaf size has been determined, the advantage of metabolic adaptation might become insignificant for these components.

CONCLUSION

In addition to the loss of lipids, thylakoid membranes experience structural alterations under drought. The chloroplast volume decreases due to a reduction in the inter-thylakoid space and in the length of agranal thylakoids. C306 plants seem to withstand drought using both morphological and physiological mechanisms to slow down or control the rate of water loss. The cultivar has the ability to maintain cell membrane integrity as well as to activate protective mechanisms that increase catalase, number of peroxisomes, Golgi bodies, ribosomes and protein biosynthesis. Alterations in plants cellular membrane composition and content during water deficit could supposedly render these membranes less efficient in enzymatic reactions, inducing precocious senescence processes and consequent loss of productivity. Inhibition of cell expansion results in a slowing of leaf expansion early in the development of water deficits and in a reduced period of grain growth. Abscisic acid accumulation induced programmed cell death and enhanced maturity. Changes in plant and cell wall composition are associated with strong perturbations in the expression of genes

involved in energetics and stress metabolism, signaling cascades and cell wall development. The number of peroxisomes increased as a result of membrane damage and consequently photorespiration was increased; therefore, there was enhanced degeneration of photosynthetic cells at different stages of plant growth and development under the water loss and higher temperature conditions to which flag leaves were subjected.

Drought-tolerant cultivar synthesized lignin around vascular bundle to avoid cavitations during rehydration under normal planting. All three different cultivars lignified whole leaf tissue to survive under drought conditions created by high temperature in January-sown plants. The vascular bundle and epidermis were separated from photosynthetic cells by ectopic expression of lignin accompanied with the formation of a glassy matrix in reverse cytogenesis studies. Abscisic acid accumulation due to high temperature did not inhibit cell division, but reduced cell expansion, leaf area, internode length, plant height, duration required for ear emergence and anthesis, spike size, fertility of spike, pollen tube length and consequently the yields.

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