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Identifying sources of water deficit tolerance on the basis of physiological and biochemical attributes in wheat introgression lines derived from chromosome 5U of wild wheat Aegilops triuncialis

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Abstract

Water deficit is a major constraint to wheat productivity, necessitating the identification of genotypes with enhanced physiological resilience and stable yield under stress. The present study aimed to identify potential sources for water deficit tolerance and elucidate the physiological and biochemical mechanisms underlying water deficit tolerance in four introgression lines (ILs: 5U-24, 5U-26, 5U-27 and 5U-31). These ILs were developed from a cross involving a disomic substitution line DS5U^{t}(5A) and wheat cultivars Pavon *ph1b* and WL711. Wheat variety PBW725 was used as check along with ILs, Pavon *ph1b* and WL711. The experiment was conducted under irrigated and rain-fed (water deficit) conditions, and the responses were evaluated at anthesis and 15 days after anthesis. Rain-fed conditions caused a significant ($p \leq 0.05$) reduction in grain yield across all lines while IL 5U-26 maintained its yield. Thousand-grain weight declined significantly in PBW725, WL711, and IL 5U-31, whereas it increased significantly in ILs 5U-24, 5U-26, and 5U-27. No significant effect was observed in Pavon *ph1b* and DS5U^{t}(5A). All lines exhibited osmotic adjustment under water deficit through accumulation of proline, glycine betaine and total soluble sugars. Water deficit stress led to increased hydrogen peroxide and malondialdehyde contents, with comparatively lower accumulation in tolerant lines. Stress conditions also reduced relative water content, canopy temperature depression, chlorophyll content, and quantum efficiency of PSII, while increasing non-photochemical quenching. These effects were more pronounced in IL 5U-31 and PBW725 at both growth stages. Stem reserve mobilization increased significantly under water deficit, with the highest enhancement observed in IL 5U-26. Principal component analysis indicated that enhanced stem reserve mobilization, higher soluble sugar content, better maintenance of relative water content, chlorophyll content and lower oxidative damage were key contributors to water deficit tolerance. Among the evaluated lines, IL 5U-26 exhibited the highest level of tolerance and can act as a potential donor for developing high-yielding, drought-resilient wheat cultivars.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal in the world because it has been domesticated and is the main staple crop worldwide (Gupta et al. 2025). Though its productivity remains inadequate, it still occupies the majority of arable land (38.8%) and has a substantially higher grain protein content (12–15%) than other cereals (FAO 2016). A range of abiotic stresses caused by climate change and global warming may cause it to decline even further. A major environmental stressor that adversely affects wheat development and productivity is water deficit, which can result in yield reductions of as much as 50% (Zhang et al. 2018; Attia et al. 2021). Agricultural water stress is increasing due to erratic rainfall and rising temperatures, decreasing groundwater resources, and increasing competition for water from commercial and domestic sectors. Rapid population growth and the expansion of irrigated agriculture have also intensified pressure on available freshwater resources, making effective water utilization in agricultural production increasingly important. In India, water scarcity is particularly severe in the north-western Indo-Gangetic Plains, including Punjab, Haryana, and western Uttar Pradesh, where excessive groundwater extraction for intensive rice-wheat cropping systems has significantly depleted aquifers. Similar water-limited conditions also occur worldwide, in major wheat-growing regions including parts of South Asia, the Middle East, North Africa, Australia, and the Mediterranean region.

Water stress leads to several physiological and biochemical changes that restrict crop growth and contribute significantly to reduction in crop productivity (Desoky et al. 2020). A decline in plant water status is one of the earliest physiological responses to water deficit, which is reflected by reduced relative water content (RWC) in plant tissues. Therefore, leaf relative water content is one of the most dependable and extensively applied markers for characterizing plant sensitivity and tolerance to water deficiency stress (Soltys-Kalina et al. 2016) Reduced tissue hydration affects physiological processes such as stomatal conductance, canopy temperature regulation, and photosynthesis. Drought-tolerant genotypes generally maintain relatively higher RWC and exhibit greater canopy temperature depression (CTD) than sensitive ones (Lepekhov 2022).

Photosynthesis is among the most sensitive processes affected by drought. Upon moderate water-stress, photosynthetic activity declines mainly due to stomatal closure, which constitutes the stomatal limitation of photosynthesis (Zahra et al. 2023). As the water-stress intensifies, biochemical limitations become more prominent, including impairment of photosynthetic enzymes and damage to the photosynthetic apparatus (Chada et al. 2023). Chlorophyll fluorescence metrics are thought to be a reliable indicator of the overall performance or stress sensitivity of genotypes and crops so they are frequently employed to evaluate the functioning of the photosynthetic machinery under drought stress (Cen et al. 2017; Kalaji et al. 2018). Drought stress generally reduces the maximum quantum efficiency of photosystem II (Fv/Fm), indicating photoinhibition, while non-photochemical quenching (NPQ) increases as a photoprotective mechanism to dissipate excess excitation energy absorbed by PSII (Guidi et al. 2019).

Besides physiological disruptions, water deficit also triggers several biochemical changes in plant cells. It induces the excessive generation of reactive oxygen species (ROS): hydrogen peroxide, singlet oxygen, hydroxyl radical and superoxide anions that may results in

oxidative stress leading to the destruction of lipids, proteins, nucleic acids and photosynthetic pigments in a cell (Sachdev et al. 2021). Also, exposure to stress may result in membrane damage and, thus, increased malondialdehyde content in the cells. To neutralize and detoxify the excessively produced ROS plants have a well-evolved system of osmotic adjustment and antioxidative defence mechanism (enzymatic and non-enzymatic) (Naik and Naik 2025). There is an increased production of osmolytes (non-enzymatic antioxidants) such as proline, glycine betaine, soluble sugars etc. under stress conditions which help maintain tissue water potential and thus allow for the maintenance of physiological activities (Kumar et al. 2024). The levels of antioxidant enzymes and osmolytes vary among species and even among genotypes of the same species, contributing to differences in drought tolerance (Patil et al. 2021). Plants adopt additional adaptive strategies to cope with drought stress. Some tolerant genotypes maintain higher membrane stability and photosynthetic activity even under reduced water potential (Bashir et al. 2021).

In wheat, grain growth depends on photosynthetic assimilates being transported from reserve pools in vegetative tissues to the grain directly (Ehdaie et al. 2008). During the grain filling, flag leaf is the principal supplier of photo-assimilates to the developing grains, thus, photosynthesis in the flag leaf has a significant effect on final grain yield production (Zhang et al. 2018). The occurrence of water-stress during this period leads to increased senescence, declined photosynthesis and reduced current photosynthetic assimilates, which in turn may reduce grain weight (thousand-grain weight) and final grain yield. In wheat, water-soluble carbohydrates primarily glucose, fructose, sucrose and fructans, build up in the stem and sheath between stem elongation and the initial phases of grain filling and these are remobilized during the later stage of grain filling (Gaur et al. 2021). According to Kaur et al. (2024), this reserve of WSC is directly linked with grain yield both during periods of irrigation as well as drought and serves as a substantial carbon source for wheat grain output. Under favourable conditions, reallocation of WSC from stem may provide about 20% of the final grain weight during grain filling, but when the crop is challenged by drought stem WSC may amount to about 50% of grain yield (Li et al. 2020). Genotypes which can withstand drought had greater WSC concentrations than susceptible ones (El Habti et al. 2020). Therefore, stem reserve dynamics represent an important adaptive mechanism for drought tolerance.

So, there is a need to develop cultivars which show stable physiological and biochemical parameters under water deficit conditions. However, due to the limited genetic base of modern bread wheat, exploiting wild relatives has become a strategic approach to broaden genetic diversity for water deficit traits. *Ae. triuncialis*, a wild progenitor species with UC genome, represents a valuable reservoir of genetic variation for abiotic stress tolerance (Badaeva et al. 2025). This species has been recognized for its adaptability to harsh environments and has potential to contribute beneficial alleles to cultivated wheat. The UC genome of *Ae. triuncialis* provides distinctive genetic resources that can be integrated into cultivated wheat via introgression breeding, hence improving resistance and productivity in water-scarce environments.

Therefore, the present study intended to identify potential sources for water deficit tolerance and the key physiological and biochemical traits associated with improved grain filling and yield stability under water deficit stress, which could be utilized in breeding programs to develop high-yielding, drought-resilient wheat cultivars.

Materials and Methods

The experimental material comprised four wheat introgression lines (ILs: 5U-24, 5U-26, 5U-27, and 5U-31), developed through interspecific introgression of chromosomal segments from *Aegilops triuncialis* into the bread wheat (*Triticum aestivum* L.) background. These ILs carry fragments of chromosome 5U derived from *Ae. triuncialis* accession pau 3549.

The ILs were generated using a disomic substitution line, DS5U^{t}(5A), in which wheat chromosome 5A is replaced by chromosome 5U of *Ae. triuncialis*, developed in the genetic background of cultivar WL711 (Sarbarzeh et al. 2002; Singh et al 2000; Sagar et al. 2018). This line was crossed with the wheat genotype Pavon *ph1b* mutant, which carries a deletion at the *Ph1b* locus enabling homoeologous recombination, thereby facilitating introgression from the wild genome. The resulting progeny were subsequently crossed with WL711, followed by selection and selfing to develop stabilized BC_1F_7 introgression lines.

The parental genotypes included WL711 (a drought-sensitive cultivar), Pavon *ph1b* mutant, and DS5U^{t}(5A), which has been reported to perform relatively better under water deficit conditions. Wheat variety PBW725 was included as a check genotype.

The field experiment was carried out at the experimental area of Punjab Agricultural University, Ludhiana, Punjab, India. Two irrigation regimes were imposed: timely irrigated (TI) and rain-fed (RF). In the TI treatment, irrigation was applied as per recommendation of PAU throughout the growing season. In the RF treatment, only a single irrigation was applied at 21 days after sowing to ensure uniform crop establishment, after which no further irrigation was provided until maturity. Consequently, the crop was exposed to natural rainfall, resulting in intermittent water deficit conditions rather than complete drought. During the cropping seasons, rainfall events (≥ 4 mm)

occurred on 6 days in 2020–21 and 8 days in 2021–22. (Supplementary Fig. 2). The experiment was conducted in two consecutive wheat growing seasons (2020-21 and 2021-22) in a randomized block design (RBD) with two replications and each genotype was sown in four rows of 1 m with row-to-row distance of 0.20 m. The meteorological data of temperature and rainfall for the both years is given in supplementary Figs. 1 and 2, respectively.

The biochemical and physiological traits were estimated from the flag leaves at anthesis and 15 days after anthesis (15 DAA) stage under both the TI and RF conditions.

Determination of biochemical attributes:

Proline was determined using the standard method of Bates et al. (1973). The fresh leaf tissue (0.1 g) was homogenised in 4 ml of 3% (w/v) sulphosalicylic acid followed by centrifugation at 3000 rpm. Two millilitres of the supernatant was reacted with acetic acid (2 ml) and ninhydrin reagent (2 ml) and then the solution incubated in a water bath at 100°C. After an hour, reaction was stopped by placing it on ice and 4 ml of toluene was added. The upper pink toluene layer was collected and absorbance at 520 nm, using toluene as blank. The proline was calculated from a standard curve and expressed as µmol g^{-1} FW.

Glycine betaine was extracted in distilled water using the standard method of Grieve and Grattan (1983). An aliquot of 0.5 ml of extract was mixed with 0.5 ml of 2 N H_2SO_4 and kept at room temperature for 2 hours. Subsequently, KI_3 solution (0.2 ml) was added and the mixture was placed in an ice bath for 90 minutes. After cooling, 6 ml of 1,2-dichloro-methane and 2.8 ml of chilled water were added and absorbance of the lower layer (red colour) was measured at 365 nm. The glycine betaine was expressed in µmol g^{-1} FW.

Hydrogen peroxide (HP) content was estimated using the protocol of Velikova et al. (2000). Fresh tissue (0.03 g) was homogenised in of 0.5% TCA (5 ml) and centrifuged at 12,000 rpm for 15 minutes. The supernatant (0.5 ml) was mixed with 0.5 ml of 50 mM potassium phosphate buffer and 1 ml of 1 M KI. The absorbance of the solution and blank was read at 390 nm and hydrogen peroxide content was estimated in µmol g^{-1} FW.

Malondialdehyde (MDA) content was estimated as per the method used by Ekmekci and Terzioglu (2005). 0.03 g of tissue was homogenised in 5 ml of 0.5% TCA and centrifuged at 12,000 rpm for about 15 minutes. 1 ml of supernatant was mixed with 1 ml of 20% TCA containing 0.5% TBA and the solution was boiled in a water bath for 30 minutes at 95°C. The reaction was terminated on ice followed by centrifugation at 10,000 rpm for 5 minutes. The absorbance was recorded at 532 nm and 600 nm and MDA content was expressed in nmol g^{-1} FW.

Total soluble sugars were estimated as per the method of Dubois et al. (1956). Fresh tissue (0.1g) was extracted twice with 80% ethanol and the pooled extracts were placed in a water bath for 2 hours to remove ethanol. An aliquot (1 ml) of extract was mixed with 5 ml of concentrated H_2SO_4 and 1 ml of 5% phenol and after 30 min, the absorbance was read at 490 nm. The total soluble sugars were expressed as mg g^{-1} FW.

Determination of physiological attributes:

Leaf relative water content (RWC) was estimated as per the standard methodology of Schonfeld et al. (1988). The leaf samples were cut into small discs of uniform size. Five leaf discs were immediately weighed to record fresh weight (FW). To estimate turgid weight (TW), another five leaf discs were immersed in distilled water at room temperature in the dark for 4–6 hours until fully turgid. After rehydration, the discs were gently blotted using filter paper and weighed to obtain the TW. Five discs were placed in hot air oven at 70°C until their weight became constant. They were weighed to record the dry weight (DW). RWC was estimated by employing the following formula:

RWC= [(FW - DW) / (TW - DW)] x 100

Canopy temperature depression (CTD) was measured using a handheld infrared thermometer (IRT) on clear days between 12:00 noon and 2.00 pm. The IRT was held 0.5–1.0 m above the canopy at an angle of 30–45° to avoid soil interference. Canopy temperature (Tc) was recorded from multiple points per plot and averaged, while ambient air temperature (Ta) was measured simultaneously at canopy height.

CTD (°C) was calculated as: CTD = Ta − Tc

Chlorophyll content was measured on the surface of the fully developed flag leaf at the mid-portion of the lamina, avoiding the midrib, using a SPAD-502 chlorophyll meter (Konica-Minolta, Tokyo, Japan). SPAD values were taken from multiple randomly selected plants from each plot and averaged to obtain a representative value. The mean SPAD value was used as an index of relative chlorophyll content.

PSII photochemistry:

Maximum quantum yield of PS II photochemistry (Fv/Fm) and non-photochemical fluorescence quenching (NPQ) was measured by using PAM meter (MONITORING-PAM; Heinz Walz, Effeltrich, Germany)

To estimate stem reserve mobilization (SRM) (%), fresh stem samples were collected at anthesis and maturity stage which were oven dried to obtain dry weight. SRM (%) was calculated by the following formula:

SRM (%) = [(DW_anthesis – DW_maturity / DW_anthesis)] x 100

Yield and yield contributing traits:

Thousand-grain weight of harvested seeds was determined by counting one thousand grains using a seed counter and weighing them with an electronic weighing balance. Grain yield was recorded by weighing the total seed weight from each plot after harvesting and threshing.

Statistical analysis

The data was analysed using three-way factorial randomized complete block design (RCBD) with two replications in Statistix 10 software. Lines, irrigation level and growth stages were the three factors used in analysis of data. Means, analysis of variance (ANOVA) and coefficient of variation were obtained to determine the effect of lines, growth stage, treatment and their subsequent interaction for all measured parameters. Principal component analysis (PCA) was carried out using package “factoextra” to generate a ggplot-2 based biplot in R-studio software to determine the relationship among the the lines and studied traits under RF conditions. Pearson’s correlation coefficients were calculated to assess the relationships among physiological, biochemical, and yield traits under stress conditions using pooled data from two years. The analysis was performed in R software (version 4.2.2) and significance of correlations was tested at $p \leq 0.05$.

Results

Biochemical attributes:

Proline, glycine betaine and total soluble sugars

Water deficit in rain-fed (RF) conditions caused a significant ($p \leq 0.05$) increase in proline and glycine betaine at anthesis and 15 DAA compared to timely irrigated conditions (TI) (Supplementary table S1). These traits varied significantly among the lines, treatments, stages and their interactions (Table 1). Parents viz. Pavon *ph1b*, WL711 and DS5U^{t}(5A) exhibited an increase in proline at anthesis and 15 DAA under RF conditions (Table 3). A similar trend was observed in check PBW725. The maximum percent increase in proline was observed in IL 5U-31 (398.3% at anthesis and 417.69% at 15 DAA), whereas 5U-24 showed minimum increase in proline at both anthesis as well as at 15 DAA (105.3% and 228.6%, respectively). For glycine betaine, the highest percent increase among the parents was observed in DS5U^{t}(5A) (52.84% at anthesis and 61.91% at 15 DAA) (Table 3). At anthesis, the percent increase of glycine betaine in 5U-26 (52.60%) was at par with DS5U^{t}(5A) while at 15 DAA it was higher (76.53%) than that of DS5U^{t}(5A). At 15 DAA, all the ILs showed significantly higher percent increase in glycine betaine than DS5U^{t}(5A).

The levels of total soluble sugars (TSS) also enhanced significantly under the RF conditions at both stages (Supplementary table S1). Among the lines, maximum increase in total soluble sugars was observed in 5U-26 (49.86% at anthesis and 50.25% at 15 DAA) whereas 5U-31 showed minimum increase (24.33% at anthesis and 16.24% at 15 DAA).

Table 1
Analysis of variance across wheat lines, treatments, growth stages and their interactions for biochemical and physiological traits as influenced by water deficit stress

S. No.	Source of variation	Proline	GB	MDA	HP	TSS	RWC	CTD	Chl C	Fv/Fm	NPQ
1	Lines	5.56*	0.54*	4.99*	3.83*	107.8*	98.67*	2.03*	115.2*	0.01*	0.00*
2	Treatments	245.2*	84.13*	662.8*	9.81*	1632.1*	7508.3*	10.10*	538.0*	0.21*	0.01*
3	Stages	13.37*	10.74*	150.52*	32.88*	7697.5*	2937.0*	1.00*	338.8*	0.28*	0.03*
4	Lines*treatments	3.304*	0.37*	1.99ns	0.19*	16.10*	123.6*	0.06*	6.28*	0.00*	0.00ns
5	Lines*stages	0.698*	0.87*	5.56*	0.53*	33.48*	35.67*	0.31*	3.22*	0.00*	0.00*
6	Treatments*stages	8.17*	2.53*	18.39*	0.91*	202.5*	68.34*	0.00ns	48.30*	0.03*	0.00*
7	Lines*treatments*stages	0.335*	0.15*	0.54*	0.02*	3.51ns	3.20*	0.01*	0.89ns	0.00*	0.00ns
8	Error	0.004	0.003	0.16	0.002	2.75	0.08	0.00	0.64	0.00	0.00

*Significant at p ≤ 0.05, ns- non-significant. Proline (Proline), GB- Glycine betaine, MDA-malondialdehyde content, HP- hydrogen peroxide content, TSS- total soluble sugars, RWC-leaf relative water content, CTD- canopy temperature depression, Chl C- chlorophyll content (Chl C), Fv/Fm- quantum efficiency of PSII and NPQ- non-photochemical quenching.

Table 2
Analysis of variance across wheat lines, treatments, growth stages and their interactions for yield and contributing traits as influenced by water deficit stress

S. No.	Source	GY	TGW	SRM
1	Lines	24557.3*	191.71*	128.52*
2	Treatments	96569.2*	000.73ns	1196.04*
3	Lines*treatments	08180.4*	021.03*	006.44*
4	Error	203.5	0.38	3.38

*Significant at p ≤ 0.05, ns- non-significant. GY- grain yield, TGW- thousand-grain weight, SRM- stem reserve mobilization.

Cell damage

The induction of water deficit was accompanied by a significant increase in MDA and HP content at both anthesis and 15 DAA (Supplementary table S1) with significant variation among the lines, treatments, stages and their interactions (Table 1). Among the parents, DS5U^{t}(5A) showed the lowest percent increase in MDA and HP content (Table 3). Among the ILs, 5U-26 exhibited the minimum percent increase in MDA and HP at both stages, while 5U-31 showed the maximum increase.

Table 3
Percent increase in biochemical parameters of wheat genotypes at anthesis and 15 DAA as influenced by water deficit conditions

S. No.	Traits	Proline		GB		TSS		MDA		HP	
	Lines	Anthesis	15 DAA	Anthesis	15 DAA	Anthesis	15 DAA	Anthesis	15 DAA	Anthesis	15 DAA
1	PBW725	327.2	413.4	58.50	92.00	30.64	33.46	79.64	86.85	39.83	41.21
2	Pavon ph1b	281.4	321.8	48.86	52.28	41.10	41.67	71.81	77.11	32.60	38.60
3	WL711	309.1	330.1	41.74	51.24	35.58	39.21	77.40	84.37	36.03	39.88
4	DS5U^{t}(5A)	345.5	406.8	52.84	61.91	40.23	44.39	64.59	74.21	31.32	36.53
5	5U-24	105.3	228.5	58.41	64.32	42.23	38.44	74.92	81.80	32.63	38.22
6	5U-26	291.2	366.7	52.60	76.53	49.86	50.25	72.02	75.73	30.92	27.08
7	5U-27	319.3	376.5	42.66	54.69	47.22	42.85	74.68	82.34	37.22	31.30
8	5U-31	398.3	417.6	97.25	95.16	24.33	16.23	80.27	87.50	45.81	47.63

Proline (Proline), GB- Glycine betaine, MDA-malondialdehyde content, HP- hydrogen peroxide content, TSS- total soluble sugars.

Physiological attributes

Leaf relative water content (RWC)

RWC indicated that all lines responded differently under TI and RF conditions and a significant ($p \leq 0.05$) decrease in the RWC was observed under RF conditions at anthesis as well as at 15 DAA stage (Supplementary table S2). RWC varied significantly ($p \leq 0.05$) among the lines, treatments, stages and their interactions (Table 1). Water deficit resulted in a significant decrease of 17.02% and 24.42% in check PBW725 at anthesis and 15 DAA, respectively, in RWC (Table 4). The percent decrease in RWC content in 5U-26 was at par with that of the parent DS5Ut(5A) at the anthesis stage. At 15 DAA, the RWC of 5U-26 was observed to be the lowest among the check, parents and other ILs. 5U-31 showed maximum percent decrease in RWC among ILs, parents and check at both the stages.

Canopy temperature depression (CTD)

Significant variation in CTD was observed among the lines, treatments, stages and their interactions (Table 1) and it was recorded to be significantly lower under RF than TI conditions at both the stages (Supplementary table S2). Also, the percent decrease in CTD in 5U-26 was lowest among check, parents and ILs (Table 4). The percent decrease in 5U-27 (22.86%) was statistically similar with parent DS5U^{t}(5A) at anthesis stage. Maximum percent decrease in CTD was observed in 5U-31 followed by PBW725.

Chlorophyll content (Chl C)

Lines, stages and their interactions varied significantly across treatments (Table 1) and Chl C decreased under RF conditions at anthesis and 15 DAA stage (Supplementary table S2). Percent decrease in Chl C was observed to be minimum in DS5U^{t}(5A) at anthesis as well as at 15 DAA among the parents (Table 4). However, IL 5U-26 exhibited minimum percent decrease in Chl C at anthesis and 15 DAA, among the parents, ILs and check PBW725. The maximum percent decrease in Chl C was exhibited by 5U-31 at both the stages.

Quantum efficiency of PSII (Fv/Fm) and non-photochemical quenching (NPQ)

Quantum efficiency of PSII (Fv/Fm) and non-photochemical quenching (NPQ) varied significantly among the ILs, parents, check, treatments and stages (Table 1) and water deficit caused a significant (significant at $p \leq 0.05$) decline in Fv/Fm while an increase in NPQ in parents, checks and ILs during the stress conditions at both the stages studied (Supplementary table S2). Among the ILs, parents and check, maximum decrease in quantum efficiency of PSII was observed in 5U-31 (Table 4) at both the stages, whereas 5U-26 reported the minimum (non-significant) decrease in quantum efficiency of PSII at anthesis (03.00%) but a significant ($p \leq 0.05$) decrease at 15 DAA (12.17%). Also, percent increase in NPQ was maximum in DS5U^{t}(5A) among the parents at both anthesis as well as at 15 DAA. The percent increase in 5U-26 at 15 DAA (15.41%) was at par with percent increase in parent DS5U^{t}(5A) at 15 DAA.

Table 4
Percent change in physiological parameters of wheat genotypes at anthesis and 15 DAA as influenced by water deficit conditions

	Traits	RWC		CTD		Chl C		Fv/Fm		NPQ	
	Percent change	Percent decrease		Percent decrease		Percent decrease		Percent decrease		Percent increase	
S. No.	Lines	Anthesis	15 DAA	Anthesis	15 DAA	Anthesis	15 DAA	Anthesis	15 DAA	Anthesis	15 DAA
1	PBW725	17.02	24.41	34.78	45.00	09.03	22.56	09.32	19.56	04.85	09.88
2	Pavon ph1b	14.58	18.51	23.47	22.26	06.47	14.44	08.24	17.75	06.61	13.65
3	WL711	15.05	23.52	26.90	28.15	09.56	14.51	06.64	15.93	06.41	13.76
4	DS5U^{t}(5A)	13.68	18.18	22.85	20.00	05.77	12.78	04.05	15.58	07.98	15.44
5	5U-24	15.95	22.78	32.00	28.57	06.78	12.94	09.05	18.75	06.06	14.57
6	5U-26	13.68	16.47	19.69	18.27	04.09	10.48	03.00	12.17	08.75	15.41
7	5U-27	14.44	17.64	22.85	26.66	08.14	14.34	05.28	17.68	07.88	13.43
8	5U-31	31.57	40.90	36.84	58.82	15.52	25.90	10.77	20.42	04.60	09.12

RWC-leaf relative water content, *CTD*- canopy temperature depression, *Chl C*- chlorophyll content, *Fv/Fm*- quantum efficiency of PSII and *NPQ*- non-photochemical quenching.

Yield and contributing traits:

Stem reserve mobilization (SRM)

Significant variation in lines, treatment and their interaction were observed for SRM (Table 2) and during stress period, SRM was increased significantly in all the ILs, parents and check (Supplementary table S3). Among the parents, maximum percent increase in SRM was recorded in DS5U^{t}(5A) (32.94%) (Table 5). The percent increase in SRM in 5U-26 (35.21%) was highest among the lines while the minimum percent increase was observed in PBW725 followed by 5U-31. The percent increase in 5U-27 was at par with parent DS5U^{t}(5A) under RF conditions.

Thousand-grain weight (TGW) and grain yield (GY)

Thousand-grain weight (TGW) and grain yield (GY) varied significantly among the lines, treatments and their interaction under both TI and RF conditions (Table 2, Supplementary table S3). Under water deficit, GY decreased in all the parents, checks and ILs except for 5U-26, which showed a slight increase in GY. The percent decrease in GY was observed to be minimum in DS5U^{t}(5A) followed by 5U-27 under RF conditions (Table 5). Among the lines, maximum percent decrease in GY was observed in Pavon *ph1b* followed by 5U-31. Check PBW725 exhibited a decrease of 39.38% in GY. TGW also decreased significantly under water deficit conditions in check PBW725, WL711 and IL 5U-31, while it increased significantly in ILs 5U-24 and 5U-27 by (Supplementary table S3) with maximum percent increase (18.15%) in IL 5U-26 (Table 5). Also, TGW decreased in parent Pavon *ph1b* by 0.67% and parent DS5U^{t}(5A) by 02.89% under water deficit conditions.

Table 5
Percent change in yield and contributing traits of wheat genotypes as influenced by water deficit conditions

S. No.	Lines	SRM	TGW	GY
1	**PBW725**	21.38	-13.34	-39.38
2	**Pavon ph1b**	32.27	00.66	-59.81
3	**WL711**	27.55	-07.64	-32.17
4	**DS5U^t(5A)**	32.94	-02.89	-08.33
5	**5U-24**	30.14	05.68	-43.33
6	**5U-26**	35.52	18.15	01.18
7	**5U-27**	32.19	10.90	-12.19
8	**5U-31**	22.42	-15.84	-55.09

*Negative values depict percent decrease while positive values represent percent increase. GY- grain yield, TGW- thousand-grain weight, SRM- stem reserve mobilization.

Principal component analysis (PCA)

PCA was performed on the evaluated dataset comprising 13 different parameters viz. proline, glycine betaine, total soluble sugars, malondialdehyde, hydrogen peroxide, leaf relative water content, canopy temperature depression, chlorophyll content, quantum efficiency of PSII (Fv/Fm), non-photochemical quenching, stem reserve mobilization, thousand-grain weight and grain yield) and 8 lines (parents, checks and ILs) with the objective of widening the discrimination in the parameters based on relationships among wheat lines under water deficit conditions. The relationship between the different wheat lines and variables with respective principal components (PCs) are further illustrated by the principal component biplots for the RF conditions at anthesis (Fig. 1A) and at 15 DAA stage (Fig. 1C).

The orthogonal transformation was defined in such a way that the first principal component has the largest variance. The first factor (PC1) determines 54.9% of the total variance of the variables and the second factor (PC2) accounts 17.8% of the variance at the anthesis stage (Fig. 1A). Together, the two PCs account for 72.17% of the variation in all the variables that were examined. The biplot analysis showed the correlation among all the examined traits. The measured parameters have a positive correlation when the angle is sharp, a negative correlation when it is obtuse and no correlation when it is right angle. PCA biplot depict sharp angle between SRM, RWC, Fv/Fm, CTD, TGW and GY indicating their strong positive associations among these traits. GY and TGW were negatively associated with HP, MDA and proline (Fig. 1A).

The first factor of PCA (PC1) analysis at 15 DAA explains 63.0% of the total variance of variables and the second factor about 15.7% of the variance (Fig. 1C). In total, both main axes explain 78.7% of the total variance of all analysed variables. PC1 separated the traits into two groups. On the positive side of PC1, Chl C, CTD, Fv/Fm, NPQ, RWC TSS SRM, TGW, GY were clustered together which indicate strong positive correlation among these traits. In contrast, Proline, MDA and HP were located on the negative side of PC1 showing negative association with physiological and yield-related traits. Further, it depicted that lines located on the positive side of PC1 [Pavon *ph1b* (2), DS5U^t(5A) (4), 5U-26 (6), and 5U-27 (7)] were associated with better physiological adaptability and yield traits. Inversely, lines located on negative side [PBW725 (1) and 5U-31 (8)] were associated with MDA and HP (oxidative stress indicators).

The Heatmap of Pearson correlation showed highly significant positive correlation between TGW and SRM [r = 0.93 (Fig. 1B and D)]. Positive and significant correlation was recorded between SRM and RWC (r = 0.75) at anthesis (Fig. 1B), SRM and TSS [r = 0.81 at anthesis (Fig. 1B) and r = 0.75 at 15 DAA (Fig. 1D)] and SRM and Chl C (r = 0.77) at anthesis. TSS and Chl C was also positively and significantly correlated [r = 0.93 at anthesis (Fig. 1B) and r = 0.88 at 15 DAA (Fig. 1D)]. Also, HP content, significantly and negatively impacted TSS [r = -0.91 at anthesis (Fig. 1B) and r = -0.76 at 15 DAA (Fig. 1D)], RWC [r = -0.93 at anthesis (Fig. 1B) and r = -0.76 at 15 DAA (Fig. 1D)] and Chl C [r = -0.97 at anthesis (Fig. 1B) and r = -0.94 at 15 DAA (Fig. 1D)].

Discussion

The present study was carried out to understand the induction of physiological and biochemical arsenal due to genome of chromosome 5U in disomic substitution line DS5U^t(5A) and its derived ILs which leads to tolerance towards water deficit conditions. The four ILs derived from disomic substitution lines contains smaller fragments of chromosome 5U while DS5U^t(5A) contain full chromosome. The

induction of water deficit around anthesis has detrimental effects on yield and its components (Mahrookashani et al. 2017). Therefore, we studied the effect of water deficit on wheat lines at anthesis as well as at 15 DAA to differentiate the physiological and biochemical response of tolerant and sensitive lines.

The increase in synthesis of different solutes in plant cell has been reported as osmoprotectant to counter the water deficit (Zulfiqar et al. 2020; Mukherjee et al. 2023). All the lines in present study exhibited osmotic adjustment through the synthesis and accumulation of compatible solutes viz. proline, glycine betaine and soluble sugars. The increase in proline, glycine betaine and soluble sugars under water deficit is the mechanism to counter osmotic stress, as these are osmoprotectants, which maintain turgor and stabilize cellular membranes (Mohammadkhani and Heidari 2008, Choudhary et al. 2022). IL 5U-24 and parents viz. Pavon *ph1b* and WL711 in present study are moderately water deficit tolerant while ILs viz. 5U-26 and 5U-27 and parent DS5U^{t}(5A) are highly water deficit tolerant and both the groups exhibited enhanced accumulation of proline, glycine betaine and soluble sugars under RF conditions. In the present study IL 5U-31 followed by check PBW725 although had higher percent increase in proline and glycine betaine but showed lower percent increase in soluble sugars as compared to other studied ILs at anthesis as well as 15 DAA stage. However, ILs, 5U-26 and 5U-27 and parent DS5U^{t}(5A) exhibited enhanced accumulation of proline, glycine betaine and soluble sugars under RF conditions, pointing towards their resilience to water deficit stress. Also, the accumulation of soluble sugars, rather than proline alone, is a more reliable indicator of effective drought tolerance, consistent with previous reports that associate higher sugar accumulation with enhanced drought resilience in wheat (Selim et al. 2019; Qayyum et al. 2021).

The degree of cell damage was observed in terms of increase in hydrogen peroxide and malondialdehyde content. Malondialdehyde is regarded as a marker for lipid peroxidation or damage to organelle membranes and plasmalemma and its levels rise with increase in environmental stress. Hydrogen peroxide is produced in plant cells under favourable conditions, but there is enhanced production under oxidative stress, caused by factors like chilling, drought, UV radiation, intense light, pathogen infection and wounding (Sharma et al. 2012). One of the reasons for increased amount of hydrogen peroxide content under stress is stomatal closure, which leads to low availability of CO_2, thereby enhancing photorespiration. In our study, the induction of water deficit was concomitant with a surge in MDA and hydrogen peroxide content with highest percent increase IL 5U-31. Oxidative damage to lipids and weakening of membrane integrity under drought conditions in wheat has been reported by Stoilova et al. (2010) and Qayyum et al. (2021). In contrast, IL 5U-26 and parent DS5U^{t}(5A) maintained lower levels of oxidative damage, suggesting more efficient antioxidant regulation and membrane stability under stress.

Leaf relative water content (RWC) is an indicator of plant water status which declined significantly under RF conditions, corroborating earlier studies in wheat (Qaseem et al. 2019). The present study indicates maintenance of RWC in IL 5U-26 and it was statistically similar with the parent DS5U^{t}(5A). The decline in relative water content may be due to reduced osmotic potential and increased transpiration in stressed leaf (Hussain et al. 2018).

Another integrative characteristic that indicates the water status of the plant is canopy temperature (Berger et al. 2010). High levels of solar radiations and water deficit conditions decrease stomatal conductance, and soil moisture which reduces transpiration rate, in turn increases canopy temperature (Rebetzke et al. 2013). Therefore, it is possible to evaluate plant's resistance to heat and drought using canopy temperature depression (difference between air temperature and canopy temperature). In the current study, canopy temperature depression decreased under RF conditions with minimum and maximum percent decrease in IL 5U-26 and 5U-31, respectively.

Chlorophyll content is one of the physiological traits sensitive to environmental stress and is the main component of photosynthesis (Hussain et al. 2019). In our study, decrease in chlorophyll content was observed at anthesis as well as at 15 DAA under RF conditions. Water deficiency induced lipid peroxidation and electrolytic leakage from thylakoid and chloroplast membranes may result in decreased chlorophyll content (Djanaguiraman et al. 2010; Wasaya et al. 2021)). Further, Fv/Fm value, which is a strong indicator of the maximum quantum yield of photosystem II (PSII) decreased under stress, reflecting photoinhibition and reduced photosynthetic efficiency. In contrast, non-photochemical quenching (NPQ) increased, particularly in 5U-26, indicating activation of photoprotective mechanisms that dissipate excess excitation energy as heat (Maxwell and Johnson 2000). The ability of IL 5U-26 and DS5U^{t}(5A) to maintain higher Fv/Fm values while enhancing NPQ at anthesis as well as at 15 DAA suggests superior protection of the photosynthetic apparatus under water deficit stress in agreement with earlier studies (Kiani et al. 2008; Chunmei et al. 2011).

Stem reserve mobilization (SRM) plays a critical role in sustaining grain filling when current photosynthesis is compromised by stress. Under water deficit conditions, stored WSCs remobilize from source to sink and this has been associated with improved yield stability in drought-tolerant wheat genotypes (Liu et al. 2020; Nazir et al. 2021). In the present study, during the stress periods, SRM was enhanced significantly in all studied ILs, parents and check, but the magnitude of increase was highest in IL 5U-26, followed by DS5U^{t}(5A) and 5U-27.

Principal component and correlation analyses further revealed strong positive associations between SRM, TGW, RWC, TSS, and chlorophyll content, underscoring the central role of carbohydrate remobilization and water status in maintaining grain weight under stress.

In our study, grain yield and TGW were significantly impacted under water deficit conditions. We observed that water deficit caused a reduction in grain yield in all ILs, parents and check variety PBW725 except for 5U-26 which was able to sustain grain yield with a concomitant and significant increase in TGW. This exceptional combination of yield stability and enhanced grain weight under stress underscores the superior water stress tolerance of this line. Similar associations between drought tolerance, assimilate remobilization, and yield stability have been reported in wheat (Bayoumi et al. 2008).

Conclusion

Thus, the present study demonstrates the functional contribution of chromosome 5U segments in conferring tolerance to water deficit stress in wheat genotypes by regulating the integrated network of physiological and biochemical traits. The efficient osmotic adjustment with balanced osmolyte accumulation, enhanced oxidative stress management, maintenance of plant water status and canopy function, protection of the photosynthetic apparatus, efficient stem reserve mobilization and yield stability even under stress highlight IL 5U-26 may act as potential genetic resource for improving tolerance to water-limited conditions in wheat.

Declarations

Ethical Approval

Not applicable

Consent to participate

Not applicable

Consent to publish

Not applicable

Competing interests

The authors declare no conflict of interest.

Funding

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Author Contribution

AS and NG conceived and designed the research. KK and NG conducted the experiments and collected the data. SS, SK and KK did the statistical analysis. KK wrote the primary draft of the manuscript. NG, AS, AK and SK reviewed and improved the manuscript.

Availability of data and materials

Not applicable

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Figures

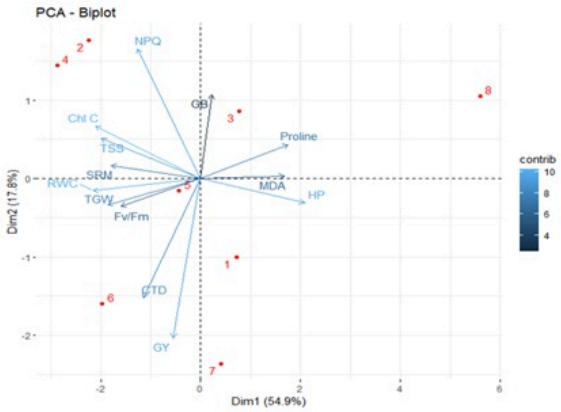


Fig. 1 A

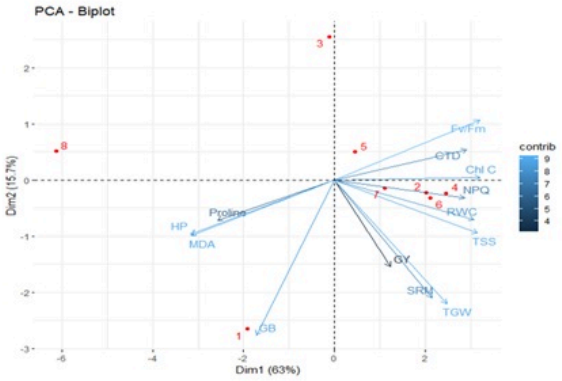


Fig. 1 C

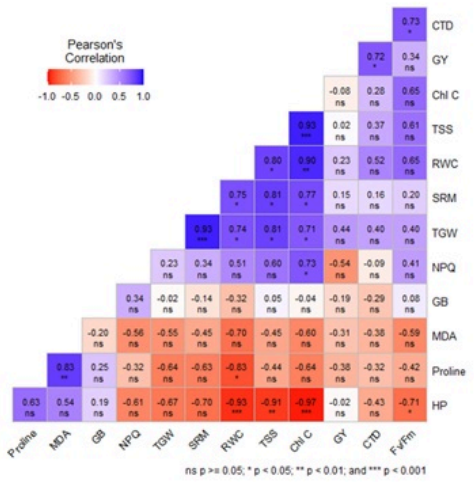


Fig. 1 B

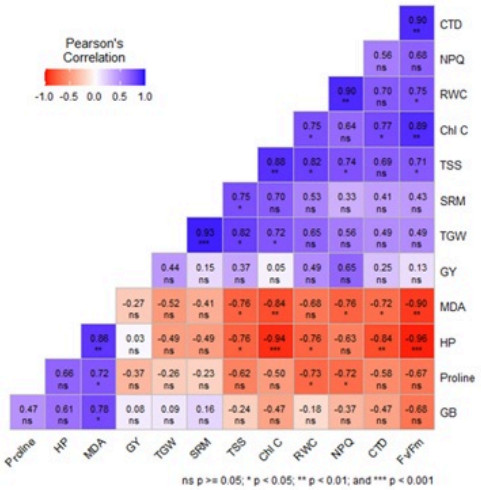


Fig. 1 D

Figure 1

A: Principal component analysis (PCA) biplot of various physiological and biochemical traits of 8 wheat lines at anthesis under rain-fed (RF) conditions, B: Heatmap of Pearson's correlation coefficient at anthesis under RF conditions, C: PCA biplot of various physiological and biochemical traits of 8 wheat lines at 15 DAA under RF conditions, D: Heatmap of Pearson's correlation coefficient at 15 DAA under RF conditions. *MDA*Malondialdehyde content, *HP* Hydrogen peroxide content, *Proline* Proline, *GB* Glycine betaine, *TSS* total soluble sugars, *Chl C*Chlorophyll content, *Fv*/*Fm* quantum yield of PSII, *NPQ*Non-photochemical quenching, *RWC* leaf relative water content, *CTD*canopy temperature depression, *SRM* stem reserve mobilization, *TGW*thousand-grain weight, *GY* grain yield

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