Phenotyping for stem reserve mobilization efficiency under heat, drought and combined stress along with defoliation in wheat (*Triticum aestivum*)

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ABSTRACT

The present study was conducted to determine genotypic variations for stem reserve mobilization efficiency in wheat (*Triticum aestivum L*) under drought, heat and combined stresses along with defoliation. Genotypes (43) were evaluated under 4 field conditions namely, timely sown irrigated (control), timely sown rainfed (drought), delayed sown irrigated (heat) and delayed sown rainfed (combined heat and drought) by cutting off all leaf blades (defoliation) at 12 days after anthesis. The traits recorded were stem reserve mobilization efficiency (SRE), harvest index (HI), grain weight (GW) and specific weight (Sp. wt). In timely sown and delayed sown environment condition the average maximum temperature was 24.7°C and 30.4°C during flowering to maturity stage respectively. The average soil moisture under control, drought, heat and combined stress was 14.46, 6.68, 16.87 and 7.78% respectively. SRE was found significantly higher under drought stress followed by combined stress, control and heat stress. The correlation analysis revealed Sp.wt at 12 DAA was highly positively correlated with the GW. The trait SRE was highly positively correlated with HI. Combined analysis for all stresses showed that HD 4728, Duram 1, Chiriya 3, HD 2851, HD 2329, DBW 43 had highest and Hindi 62, WL 711, GCP 23, HD 2967, GCP 2, Kalyansona had lowest SRE. Genotypes were also grouped into different clusters based on their SRE. The genotypes with higher SRE can be used in breeding programmes or directly used as cultivars under drought, heat and combined stress conditions.

Key words: Combined stress, Defoliation, Drought stress, Heat stress, Stem reserve mobilization efficiency

Bringing drought and heat adaptive traits together in one wheat (*Triticum aestivum L.*) genotype could increase its yields particularly in low yielding environments (Lopes et al. 2014). Sometimes crops get exposed to both heat and drought stress that occur in the field simultaneously (Shah and Paulsen 2003). The grain-filling rate in cereals is dependent on current assimilates from photosynthesis and reserve carbohydrates transported to the grain from vegetative tissues in leaves, stem and ear (Plaut et al. 2004). Stem reserve mobilization supports grain filling under stress conditions (Blum et al. 1994). The capacity of the plant to store WSC in the stems and to later remobilise these to increase grain yield has been proposed as an adaptive trait in a conceptual model for drought tolerance. The contribution of remobilised stem WSC to grain weight can increase from 30–50% under stress conditions (Zamani et al. 2014). The defoliation treatment simulates a drought stress by inhibiting current assimilation (Blum et al. 1983).

Under stress conditions, stem reserve mobilization (SRM) may contribute to 70% of the grain yield. Heat stress and limited water availability significantly impair photosynthesis and thus reduces the amount of current assimilates available to the grain. The contributions of stem and sheath assimilate reserves to grain weight/spike were from 24–84% in defoliated plants (Dodig et al. 2006). SRM enhances at high temperatures during wheat grain filling. In wheat, much of the pre-anthesis carbohydrates (largely fructans) stored in stem and sheaths can be reallocated (70–92%) to the grain under post-anthesis drought stress (Yang et al. 2001). The main objective was to identify genotypes for higher SRE in the different groups of genotypes by assessing contributing pre-anthesis stem reserves associated with the grain development in wheat.

MATERIALS AND METHODS

Growing conditions and plant materials

The field trials were carried out at ICAR-Indian Agricultural Research Institute, New Delhi, India (28°41’ North latitude and 77°01’ East longitude, 228 m AMSL). The study material comprised 43 genotypes of wheat from 7 groups, viz. recombinant inbred lines (RILs) consisting of 11 entries (9 + 2 parents), contrasting heat stress genotypes...
consisting of 12 entries (6 tolerant + 6 susceptible), contrasting drought stress genotypes consisting of 14 entries (7 tolerant + 7 susceptible), contrasting height genotypes consisting of 10 entries (5 tall + 5 dwarf), current best standard genotypes (best-adapted varieties) 6 entries, popular ruling varieties of last century consisting of 10 entries and genotypes with duration of maturity consisting of 8 entries (4 early + 4 late). Some of the entries are common in one or the other group. All these genotypes were evaluated under 4 field conditions namely, timely sown irrigated (control), timely sown rainfed (drought), delayed sown irrigated (heat) and delayed sown rainfed (combined heat and drought) by cutting off all leaf blades 12 days after anthesis (DAA) during 2016–17.

Each genotype was planted manually having gross plot size of 0.46 m × 2.5 m, with rows 23 cm apart. The timely and late sown were on 8 November, 2016 and 8 December, 2016 respectively. The date of sowing was late or delayed with time gap of 45 days to get terminal heat stress and combined heat and drought stress. The standard cultivation practices prescribed for wheat under irrigated conditions were followed precisely.

Measurements

Weather during crop season: Daily temperature, sunshine hours, evapo-transpiration, wind speed and rainfall (Table 1) were measured during wheat crop growing season for the period 2016–17.

Soil moisture: Soil samples from each experimental unit were collected in aluminium boxes with secure lids every week at 3 depths (15, 30 and 45 cm) by using augers. The samples were weighed immediately and then oven-dried at 105°C for 72 h for determining soil moisture content by gravimetric method, and it has correlated with neutron moisture meter readings. The soil moisture, temperature and precipitation during flowering to maturity stage are shown in Fig 1-2. Date of flowering started 78 and 68 days after sowing in timely delayed and sown condition respectively. The average soil moisture during wheat growing season has been depicted in Table 2.

Observations and analyses

The physiological traits including anthesis date, stem height (SH), stem specific weight (Sp.wt), harvest index (HI), stem reserve mobilization (SRM), stem reserve mobilization efficiency (SRE), grain weight percentage (GWP) and grain weight/ear (GW). Six uniform plants flowering on the same day from interior rows within each plot were tagged for sampling. Three tagged plants were sampled at 12 d after anthesis (12 DAA) and remaining 3 plants at physiological maturity (3/}

| Weather parameter               | Timely sown | Late sown |
|--------------------------------|-------------|-----------|
| Maximum temperature (°C)       | 31.0        | 38.5      |
| Minimum temperature (°C)       | 15.4        | 22.4      |
| Average maximum temperature (°C)| 24.71       | 30.35     |
| Average sunshine (h/day)       | 6.90        | 8.04      |
| Average evapotranspiration (mm)/day | 4.20    | 5.57      |
| Average AWS (kmph/day)         | 4.87        | 6.00      |
| Total rainfall (mm)            | 79.7        | 27.9      |
PHENOTYPING FOR STEM RESERVE MOBILIZATION EFFICIENCY IN WHEAT

All observations and measurements were based on the main stem. Data for traits were measured after leaf blade removal. Days to anthesis and maturity were estimated from date of sowing to 50% anther extrusion respectively. The samples of physiological maturity were collected when the flag leaf and spike turned yellow, i.e. SCMR and NDVI readings were showing in the range of 2–5 and 0.15–0.20 respectively. The SPAD chlorophyll meter reading (SCMR) was measured. The normalized differential vegetation index (NDVI) was measured using a hand-held Ntech Greenseeker (Field portable NDVI sensor).

The leaf blades were cut off at 12 DAA, around the start of the rapid grain filling phase under all the field conditions in order to know stem reserve mobilization by inhibiting the current photosynthesis. The stem weight of main stem and its ear (above ground biomass) were measured at 12 DAA and at full maturity, after plant material had been oven dried at 70°C for 48 h. Stem specific weight (Sp.wt) was calculated as the ratio of its dry weight to its length at 10 DAA. The main stem grain weight (GW) was taken after harvesting. The amount of stem reserves is measured either by changes in stem dry weight (indirect method) or by stem WSC (Water soluble carbohydrates) content (direct method) (Ehdaie et al. 2008). The amount of mobilized dry matter from stem to grain SRM was estimated as the difference between stem weight at 12 DAA and maturity. SRE was estimated as the proportional contribution (%) of mobilized stem reserve to stem weight at 12 DAA (Ehdaie et al. 2006). The harvest index (HI) was calculated as the proportional contribution (%) of economic yield to its biological yield (defoliated). Grain weight percentage (GWP) was calculated as the proportional contribution of SRM to the main stem grain weight.

Experimental design and statistical analysis

The field experiment was laid out in alpha lattice design with two replications. Analysis of variance was performed for each character. The data were analysed using the SAS (version 9.0) for ANOVA and cluster analysis while, dendrogram using centroid linkage in SPSS (version 16.0, SPSS, Chicago, IL, USA) and biplot analysis using R statistical analysis packages. Mean comparisons were done using the least significant difference (LSD) test (P<0.05). Associations between characters were examined by Pearson’s correlation test. Figures were created using Microsoft Excel 2010 (Fig 3 A–D).

### Table 2

| Soil moisture | Timely sown (Control) | Late sown (Drought stress) | Timely sown (Heat stress) | Delayed sown (Combined stress) |
|---------------|-----------------------|-----------------------------|---------------------------|-------------------------------|
| Average soil moisture (%) | 14.46 | 6.68 | 16.87 | 7.78 |

Fig 3  The relationship between specific weight (sp.wt) at 12 DAA and grain weight (GW) under Control (A), Drought stress (B), Heat stress (C) and Combined stress (D).
RESULTS AND DISCUSSION

Genotypic variation in stem reserve mobilization efficiency

Combined analysis for all stresses showed that HD 4728, Duram 1, Chiriya 3, HD 2851, HD 2329, DBW 43 and Hindi 62, WL 711, GCP 23, HD 2967, GCP 2, Kalyan Sona genotypes had highest and lowest SRE with 32.75, 29.95, 29.65, 26.37, 26.08 and 8.06, 11.50, 11.91, 12.65, 12.68, 12.75 respectively. Nevertheless, what is important is the existence of genetic variability in wheat for both the capacity to accumulate reserves in the stem and the remobilization efficiency (Ehdaie et al. 2008), which allows these traits to be improved in new genotypes.

Effect of stress along with defoliation on mobilization efficiency

The mean SRE of 43 genotypes under control, drought, heat and combined stress was 20.02, 24.95, 14.63 and 21.11 respectively. The SRE under the heat stress was very low as compared to the other stresses as high temperature adversely impacted grain formation and could not transfer the stem reserves (Fig 4). Heat stress under late sown resulted accelerated pollen mortality due to deprived starch synthesis and mobilisation, which in turn affected the final grain yield (Dwivedi et al. 2017). The levels of stem reserves were higher in water-stressed wheat plants than that in irrigated trials (Ruuska et al. 2006).

High temperature induced obstacle in starch mobilisation within the anther, which interrupts pollen development and intensifies pollen mortality (Zhang et al. 2012). Grain yield in crops is critically dependent on successful reproductive development, and evaluating pollen viability may be considered as an important criterion in selecting heat tolerant genotype (Mesihovic et al. 2016).

The principal component analysis revealed PC 1 and PC 2 accounts for 62.09% of total variation. All the genotypes near the centre of axis are comparatively more stable genotypes than those far from the axis. This also revealed highest SRE under drought stress followed by combined stress, control and heat stress. The combined analysis of all the field condition showed that highest and lowest SRE genotypes were opposite to each other in the biplot of the principle component analysis. The highest SRE genotypes fell under the PC 1 and show more favourable genotypes according to different stress conditions, viz. 26. HD 4728; 10. Duram 1; 33. Chiriya 3; 36. HD 2851; 35. HD 2329; 32. DBW 43. The lowest SRE genotypes fall under the PC2, viz. 8. Hindi 62; 4. WL 711; 30. GCP 23; 18. HD 2967; 25. GCP 2; 31. Kalyan Sona. The defoliation treatment simulates a drought stress by inhibiting current assimilation (Blum et al. 1986). Post anthesis changes in stem dry weight could also be used to estimate stem reserves and related characters instead of measuring stem WSC content which is time consuming and expensive (Ehdaie et al. 2008).

SRE of all other environmental stresses except the heat stress were higher. It was also same in GWP and HI physiological parameters.

The maximum stem weight and carbohydrate concentration occurs 10–14 days after anthesis and it is followed by a rapid decline in carbohydrate concentration during grain filling (Pierre et al. 2010). In another study, Ehdaie et al. (2006) also suggested that the potential for stem reserve accumulation depends on both stem length and stem specific weight (stem weight/stem length). Thus, the capacity for WSC reserve would increase with longer stems and greater specific weight. In addition, selection for a higher WSC at anthesis may bring negative consequences in terms of yield potential and adaptation to mild water stress conditions (Alejandro et al. 2016).

Clustering of genotypes using dendrogram centroid linkage

The clustering of the genotypes for stem remobilization efficiency under different stresses was done to identify the genotypes with higher SRE (Fig 5). Genotypes were grouped into 5 clusters of which, the highest SRE cluster consisted of 3 genotypes (HD 4728, Chiriya 3 and Duram 1) comes in

Table 4 The top five genotypes among 43 studied for SRE under different stresses

| Defoliated control | Defoliated drought stress | Defoliated heat stress | Defoliated combined stress |
|--------------------|---------------------------|------------------------|---------------------------|
| Genotypes          | SRE (%)                   | Genotypes              | SRE (%)                   |
| Duram 1            | 35.22                     | HD 2329                | 34.21                     |
| Chiriya 3          | 35.30                     | GCP 30                 | 35.03                     |
| HD 2851            | 35.49                     | Chiriya 3              | 36.78                     |
| WL 711             | 35.80                     | Westonia               | 37.26                     |
| HD 4728            | 39.29                     | PBW 343                | 46.45                     |
| Chiriya 7          | 26.41                     | Dha. Dry               | 29.51                     |
| HD 2851            | 27.68                     | PBW 343                | 30.77                     |
| WR 544             | 28.63                     | NP 4                   | 32.43                     |
| Duram 1            | 28.86                     | Chiriya 3              | 34.83                     |
| DBW 43             | 30.85                     | HD 4728                | 35.82                     |
the final 2 clusters. These are the promising lines for higher stem reserve mobilization efficiency. Grouping of genotypes into 5 distinct clusters also indicated the presence of high genetic diversity among materials tested and can be useful for using in the crop improvement program.

**Correlation and regression of the studied traits under different stresses**

The stem specific weight of stem at 12 DAA was strongly correlated with main stem grain weight under all the environmental conditions. The stem weight at 12 DAA was directly proportional to the final grain weight.

The SRE was significantly higher under drought field condition followed by combined stress, control and heat stress condition. SRE is significantly negatively correlated with Sp.wt (0.05 level) under irrigated, drought as well as combined stress field conditions. SRE is significantly positively correlated with HI (0.01 level) under control, heat and combined stress field condition. SRE also significantly negatively and positively correlated with GW (0.05 level) under control and heat stress field condition respectively.

SRE is significantly negatively correlated with Ht of a stem (0.01 level) under irrigated as well as combined heat and drought field condition (Table 5). GW is significantly positively highly correlated with Sp.wt (0.01 level) under all the stresses (r= 0.648) (Fig 4 B). The GW with Sp.wt under control, drought, heat and field condition are r = 0.793, r = 0.626, r = 0.362 and r = 0.641 respectively (Fig 3). The analysis of variance (ANOVA) revealed significant genotypic variances for all traits studied. There was high variation among genotypes for all these traits, while genotypes with high SRE were identified. Stem reserve contributes in mobilizing WSC from source to sink. Dreccer et al. (2013) indicated that stem reserves can contribute about 20–40% of the final grain weight in non-stressed environments and this can be up to 70% under stressed conditions during grain filling stage (Ehdaie et al. 2006). Ehdaie et al. (2006) suggested that the potential for stem reserve accumulation depends on both stem length and stem specific weight. Thus, the capacity for WSC reserve would increase with longer stems and greater specific weight.

The subjectivity of stem solidness scoring and the variation in the expression of the character owing to environmental conditions were identified as important factors that complicate breeding for high yielding lines with solid stems (Lanning et al. 2006). In addition, the contribution of pre-anthesis reserves was also evident in wheat cultivar LOK-1 from both defoliation and WSC experiments as reported by Mahesh Kumar et al. (2017). It is largely accepted that stem reserve mobilization in grains is affected by sink size, environment and variety. Mobilization efficiency and sink strength (number of grains/spike and grain weight) are two component traits involved in the extent of contribution of stored reserves to grain yield in wheat (Ehdaie et al. 2006).

Correlation and regression of the studied traits under different stresses

| Parameters | Defoliated control | Defoliated drought stress | Defoliated heat stress | Defoliated combined stress |
|------------|--------------------|--------------------------|------------------------|---------------------------|
| SRE (%)    | 20.02 (-0.89 to 39.29) | 24.95 (2.41 to 46.45) | 14.63 (-2.80 to 30.85) | 21.10 (3.00-35.82)       |
| GW (g/ear) | 2.35 (1.55 to 3.17)   | 2.37 (1.46 to 3.26) | 1.59 (0.98 to 2.32) | 1.81 (1.31 to 2.40)       |
| Sp. wt (mg/cm) | 17.40 (12.43 to 22.10) | 16.53 (12.80 to 21.04) | 14.54 (11.26 to 18.52) | 14.94 (11.95 to 19.44)   |
| GWP (%)    | 19.88 (0.36 to 48.16) | 21.94 (2.10 to 48.26) | 15.37 (-0.65 to 38.28) | 18.56 (0.76 to 39.75)     |

**Table 6** Pearson correlation co-efficient between stem reserve mobilization efficiency under different field conditions and physiological parameters

| Parameters | SRE in control | SRE in drought stress | SRE in heat stress | SRE in combined stress |
|------------|----------------|-----------------------|-------------------|------------------------|
| Sp.wt      | -0.352*        | -0.364*               | -0.216ns          | -0.327*                |
| HI         | 0.321*         | 0.172ns               | 0.467**           | 0.556**                |
| GW         | -0.355*        | ns                    | 0.335*            | ns                     |

ns: Non significant; * significant at P< 0.05; ** significant at P< 0.01; N= 43.
Conclusions

The present study indicated that the critical role stem reserve efficiency plays in improving yield under drought and heat stress in wheat genotypes. Physiological parameters and yield potential of wheat crop are highly influenced by different stresses along with defoliation. It was concluded that GW is highly associated with stem specific weight at 12 DAA. The SRE was found to be correlated with the HI. The study also validated that the genotypes with higher mobilization of stem reserves under different stress conditions maintained their grain yield. The duram genotypes showed higher SRE than Triticum aestivum. The genotypes screened in this experiment can be used as drought, heat and combined stress tolerance promising lines. Overall, the study provided a comprehensive understanding of stem reserve mobilization in wheat genotypes.

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