# Morpho-physiological characterization of wheat (*Triticum aestivum*) for Stay Green trait

Naresh Pratap Singh[1],Vaishali[2], Pooran Chand[3], Anil Sirohi[4], Mukesh Kumar[5], Jitender Singh[6]
[1,2,3,5]*Department of Biotechnology, SVP University of Agriculture and Technology, Meerut, India.*
[4,6]*College of Biotechnology, SVP University of Agriculture and Technology, Meerut, India.*

***Abstract*- Wheat is the major cereal crop all over the world, as the ecological cycle is changing day by day due to environmental changes so, efforts should be made to develop improved crops. Stay Green trait is one of the major character of the crop to sustain under abiotic stresses. In the present study, 10 wheat varieties viz. HUW 510, C 306, Sonalika, HD 2135, HD 2177, VL 401, K 9162, RAJ 3765, K 68, K 7410 were collected to develop the Stay Green genotype. The seeds of these ten wheat varieties were treated with 1% EMS for 90 minutes. Among the morphological characteristics of wheat varieties under controlled and treatment, the characters as: plant height, leaf area, numbers of productive tillers/plant, days of maturity were recorded at pre-harvest stage and seeds per spike, length of spike, spikelets/spike and 1000 grain weight were recorded at post-harvest stage. In the present study, significant reduction in yield components like seeds per spike, 1000 grain weight was observed in all the ten wheat varieties in control and treatment. After EMS treatment, the wheat variety K 7410 and RAJ 3765, performed better after EMS treatment can be considered as Stay Green genotype and may be resistant to abiotic stress like drought, high temperature etc.**

***Index Terms*- Wheat, stay green trait, morphological parameters, physiological characters.**

## I. INTRODUCTION

In last few years, world climatic conditions have been drastically changed. Most part of the world is under low water availability especially in South Asia and Africa. In India, 29% of the total cultivable area faces drought condition out of which 10% is under severe drought (**Anonymous 2003**). Drought and water shortages threaten the agricultural productivity of many developing countries, especially in South Asia and Africa, to feed their ever growing population. In addition Abiotic stresses such as extreme temperature, drought, heat, salinity, mineral deficiency and toxicity are frequently encountered by plants in both natural and agricultural systems. Across a range of cropping systems around the world, abiotic stresses are estimated to reduce yields to less than a half of that possible under ideal growth conditions. In addition abiotic stress reactions, especially to water deficiency and high level of salts, are complex morphological and physiological phenomenon in plants (**Wang *et al.*, 2003**). Drought stress not only affects the morphology but also severely affects the metabolism of the plant, physiology and biochemical responses in plants. These responses include stomatal closure, repression of cell growth and photosynthesis and activation of respiration. It is a permanent constraint to agricultural production in many developing countries and an occasional cause of losses of agricultural production in developed ones (**Ceccarelli and Grando 1996**). Therefore, physiological and biological approaches have a great importance in order to understand the complex responses of plants to water deficiency and to develop new varieties. When water is not a limiting factor, *Triticum* productions with late sowing (with high temperatures in the end of the cycle) have lower yields, mostly as a result of heat stress during grain filling (**Maçãs *et al.*, 2000**). It has been reported (**Sofield *et al.*, 1977**) that, during grain filling, increasing temperatures (between 15/10ºC and 21/16ºC day/night temperatures) counter balances the diminished growth duration, augmenting the filling rate. **Caley *et al.* (1990) and Jenner (1994)** further confirmed related data. **Shpiler and Blum (1986)**, working with field trials, also reported that grain maturity develops earlier, producing smaller and shriveled grains, in heat stressed genotypes. Photosynthetic activity is sensitive to high

temperature. High temperature increases the radiant energy absorbed by the leaf and, as a consequence, the process of photosynthesis is affected (**Krause and Santarius 1975; Seeman *et al.,* 1987**). Therefore, a visible trait such as leaf chlorophyll content can be used along with chlorophyll fluorescence for screening. These processes directly affect photosynthesis and, ultimately, yield. The `stay green' phenotype can arise in different ways One approach to achieve a longer photosynthetic period is the use of functional stay-green phenotypes, which show either a delayed onset or a slower rate of senescence with maintaining photosynthetic activity. In cells, senescence-related changes are first detected in the chloroplast (**Dodge 1970**). Plants with the 'stay green' trait have been shown to resist drought induced premature plant senescence (**Xu *et al.,* 2000**). Many mutations, referred to as stay green or non-yellowing in various plant species have been reported to maintain leaf greenness after the grain-ripening stage (**Fang *et al.,* 1998; Spano *et al.,* 2003; Walulu *et al.,* 1994**). Some 'stay green' mutants can photosynthesize longer and might therefore be expected to give a higher yield; these may be defined as 'functional stay green' mutants.

## II. MATERIAL AND METHODS

The present research work was carried out at laboratory of Department of Biotechnology, College of Agriculture, of S.V.P. University of Agriculture and Technology, Meerut during rabi season. A total of ten varieties of wheat cultivars viz. HUW 510, C 306, Sonalika, HD 2135, HD 2177, VL 401, K 9162, RAJ 3765, K 68, K 7410 were collected to study the effect of EMS treatment on morpho-pysiological characters. The seeds of all ten varieties were treated with EMS (1% in distill water) for 90 min in petri plates. Thereafter the treated seeds of wheat were sown in pots.

## III. MORPHOLOGICAL EVALUATION OF WHEAT VARIETIES

The data was observed by randomly selecting five plants from each varieties.The data was observed for pre-harvest characters like Plant Height(total height of plant), Number of productive tiller, Leaf Area (of flag leaf), Days of Maturity (days from sowing to the 98-100% loss of total chlorophyll content of plant).For post-harvest characters the data was recorded for Length of Spike (Ear length is measured in cm.), Number of spikelets per Spike (mean of 5 spikes per genotypes is considered for analysis), Seeds per Spike(Mean of seeds counted from 5 randomly sampled spikes at maturity is recorded for analysis), thousand Grain Weight (in gm.) were recorded from control and treatment plants for comparative study.

## IV. Physiological evaluation of wheat varieties

After inducing mutation by EMS for developing stay green trait the data for Related Water Content(RWC), Chlorophyll content and Photosynthetic rate was recorded. The RWC was measured on the wheat seedling organs following the method of **Turner (1981).** Fresh weight (FW) of flag leaf was determined immediately after harvest, and then tissue were allowed to float in distilled water until fully rehydrated. The wheat organs were weighted for turgid weight (TW). The turgid organs were dried in a hot oven at 80 °C to constant weight, and dry weight (DW) was recorded. The RWC was calculated as: RWC (%) = (FW – DW) / (TW – DW) * 100.

Chlorophyll meter (SPAD 520) were used to measures the relative chlorophyll content(µgm/$cm^2$) of the leaves and Infra Red Gas Analyser (IRGA) was used to measure the photosynthetic rate of leaves(µmol/$m^2$sec). Five readings were taken from five plant leaves of same variety and their average was considered for determination of RWC, Chlorophyll content and Photosynthetic rate.

## V. Results and Discussion

In the present investigation, attempts were made to characterize the stay green trait in some of the wheat genotypes based on the morphological and physiological characters in control and after EMS treatment (1% for 1.30hrs.).

## VI. Morphological characterization of wheat varieties under control and treated condition

### Pre-harvest characteristics

Plant height was recorded at final stage of maturity. The plant height was found to be increased after the treatment of EMS (Table 1, Figure 1). In control plants Sonalika and RAJ 3765 varieties showed higher value 96.15cm but after treatment Sonalika showed highest plant height 98.17cm. Whereas, variety K 7410 and RAJ 3765 showed decrement in plant height. the number of productive tillers per plant was varied from 9.58 in K 7410 to

7.47 in HD 2177 in controlled plants The number of productive tillers decreases after all treatments except in K 7410 and RAJ 3765 variety. Leaf area of flag leaf is directly related to higher photosynthesis and high chlorophyll content. The leaf area of flag leaf in controlled plants was varied from 34.17 to 24.03cm$^2$ in variety K 7410 and C 306. Total leaf area of flag leaf of wheat varieties was reduced significantly after EMS treatment except in variety HUW 510, K 7410 and RAJ 3765. Overall the varieties took 133 to 145 days to get mature. After EMS treatment, days to maturity increased as compared to control. Variety K 7410 and RAJ 3765 took lesser time to get mature after EMS treatment so, it could be considered as stay green genotype. On the other hand, the longest time taken by variety HD 2135 in control i.e. 145 days and 147days after the EMS treatment.

## VII. POST-HARVEST CHARACTERISTICS

In the present study, the significant variations were reported in 1000 grain weight and numbers of grains per spike amongst different varieties after treatment of EMS. The length of spike varied from 12.33cm in K 7410 to 9.67cm in HD 2177 variety in control plants but after treatment of EMS the spike length reduces significantly except in variety K 7410 (Table 2, Figure 2). The number of spiklets/spike varied from 25.12 in K 7410 to 17.63 in HD 2177 in control plants. The number of spiklets/spikes in most of the varieties reduced after the treatment of EMS except in variety K 7410 and RAJ 3765. Seeds per spike is direct measure of yield/plant, hence it is economically important post harvest characteristic. The number of seeds per spike was decreasing after EMS treatments in almost all the varieties except in variety K 7410 and RAJ 3756, from 63.17 to 64.85 and 62.07 to 62.67 respectively. 1000 Grain Weight showed the actual yield of the crop in the field. A significant reduction in test weight was observed after treatment of EMS.The weight of 1000 grain varied from higher value of 60.50g in K 7410 variety to lower value of 42.20g in VL 401 variety in control plants. The variety K 7410 and RAJ 3765 showed significantly increment in test weight in comparison to other varieties after the treatment.

This type of results suggests that they can perform better under abiotic stress conditions. This was in accordance to the reports of, **Bhullar and Jenner (1983)** who suggested that high temperature stress had a direct effect on the developing kernels. Genotypes with good tolerance to high temperature have a stable or long duration of photosynthetic activity (**Al-Khatib and Paulsen 1990).** Extending the duration of photosynthesis is a possible means to increase total photosynthesis, biomass and yield **(Richards, 2000).** Under environmental stress, e.g. high temperatures after anthesis, grain yield reduced due to a decline of single grain weight **(Porter and Gawith 1999).**

## VIII. Physiological characterization of wheat varieties under control and treatment condition

The Relative water content (RWC) will help the plant to withhold water and supply to plant for its normal functioning in unfavorable conditions. The RWC in controlled plants varied from 62.30% in K 7410 to 26.29% in Sonalika. The increased RWC after EMS treatment was observed in variety K 7410 and RAJ 3765 from 62.30 to 62.91% and from 60.69 to 61.73% and thus could be considered as stay green genotype (Table 3, Figure 3). Similarly results were coated by **Ganji *et al*. (2012),** where they found that drought conditions significantly affects RWC of wheat varieties. In other words, plant having higher yields under drought and heat stress should have high RWC. The chlorophyll content is one of the essential parameter among all of the observed parameters and can be considered as indicator of stay green genotypes. Chlorophyll content was found to be highest in K 7410 and lowest in 40.10 in HD 2135 in control. The chlorophyll content of variety K 7410 is increases significantly after the treatment of EMS from 52.58 to 54.83µg/cm$^2$. Rest of the variety showed significant decrement in chlorophyll content after the treatment. The photosynthesis rate was varied from 16.40µmol/m$^2$sec in C 306 to 23.43µmol/m$^2$sec in K 7410 in control. The photosynthesis rate was found to be increased in K 7410 and RAJ 3765 from 23.43 to 24.00µmol/m$^2$sec and from 22.57 to 23.57µmol/m$^2$sec respectively after the treatment of EMS but showed a significant reduction in photosynthetic rate after the treatment(Figure 3). Such profile of photosynthesis rate indicates that variety K 7410 and RAJ 3765 could be considered as stay green genotypes. Extending the duration of photosynthesis is a possible means to increase total photosynthesis, biomass and yield **(Richards 2000).** Total flag leaf

photosynthesis, chlorophyll content, the onset of senescence (at low nitrogen availability), and green leaf duration have all been found to be positively correlated with wheat grain yield **(Wang *et al.*, 2008; Gaju *et al.*, 2011).** Increased chlorophyll content leads to increase in yield showed by K 7410 and RAJ 3765 after three treatments of EMS. **Khayatnezhad *et al*. (2011)** reported similar results and concluded that the genotypes with high leaf chlorophyll content are tolerant to stress conditions. Stay-green phenotypes and broader genetic variation in senescence have been reported in hexaploid wheat **(Verma *et al.*, 2004; Naruoka *et al.*, 2012).** The stay-green trait has been reported to increase yields **(Christopher *et al.*, 2008; Chen *et al.*, 2010).**

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## Table 1: Pre-harvest characteristics

| S.N. | Genotypes | Plant Height (cm) | | Leaf Area(cm)$^2$ | | No. of productive Tiller | | Days To Maturity | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | Treatment | Control | Treatment | Control | Treatment | Control | Treatment |
| 1 | **HUW 510** | 86.33 | 93.50 | 29.8 | 30.77 | 9.37 | 8.83 | 143 | 145 |
| 2 | **C 306** | 87.25 | 92.08 | 24.03 | 22.87 | 8.23 | 6.30 | 143 | 145 |
| 3 | **Sonalika** | 96.15 | 98.17 | 25.13 | 23.26 | 7.58 | 6.17 | 136 | 139 |
| 4 | **HD 2135** | 86.67 | 90.67 | 26 | 24.37 | 8.17 | 6.83 | 145 | 147 |
| 5 | **HD 2177** | 81.57 | 87.58 | 25.1 | 23.67 | 7.47 | 7.25 | 140 | 143 |
| 6 | **VL 401** | 90.17 | 93.17 | 27.97 | 24.24 | 8.50 | 7.67 | 139 | 144 |
| 7 | **K 9162** | 89.13 | 92.17 | 25.03 | 22.48 | 8.52 | 7.50 | 143 | 145 |
| 8 | **RAJ 3765** | 96.15 | 91.00 | 32.47 | 33.73 | 9.37 | 10.08 | 139 | 134 |
| 9 | **K 68** | 88.98 | 94.83 | 30.03 | 27.24 | 8.42 | 6.72 | 141 | 143 |
| 10 | **K 7410** | 86.67 | 84.42 | 34.17 | 36.03 | 9.58 | 10.13 | 133 | 132 |

**C=Control, T=Treatment (1% for 1.30hrs of EMS)**

**Table 2: Post-harvest characteristics**

| S.N. | Genotypes | Length of Spike(cm) | | Spikelets/spike | | Seeds/Spikes | | 1000 Grain weight (gm) | |
|---|---|---|---|---|---|---|---|---|---|
| | | C | T | C | T | C | T | C | T |
| 1 | HUW 510 | 10.30 | 9.37 | 18 | 16.67 | 46.33 | 45.34 | 47.50 | 46.34 |
| 2 | C 306 | 10.67 | 9.80 | 20.33 | 17.67 | 49.5 | 47.67 | 42.70 | 40.30 |
| 3 | Sonalika | 10.90 | 7.50 | 20.67 | 18.33 | 50.33 | 46.33 | 44.00 | 39.80 |
| 4 | HD 2135 | 10.03 | 10.53 | 21.67 | 19.67 | 51.37 | 48.33 | 45.20 | 42.10 |
| 5 | HD 2177 | 9.67 | 8.70 | 17.63 | 15.67 | 45.84 | 40.67 | 46.30 | 42.80 |
| 6 | VL 401 | 10.13 | 9.30 | 22.67 | 21.67 | 56.67 | 53 | 42.20 | 42.13 |
| 7 | K 9162 | 10.80 | 11.47 | 21.67 | 18.67 | 50.33 | 45.78 | 47.70 | 44.07 |
| 8 | RAJ 3765 | 12.08 | 12.37 | 24.43 | 24.83 | 62.07 | 62.67 | 56.89 | 57.97 |
| 9 | K 68 | 10.93 | 10.83 | 22 | 20.67 | 58.33 | 54.67 | 50.20 | 47.23 |
| 10 | K 7410 | 12.33 | 12.90 | 25.12 | 25.78 | 63.17 | 64.85 | 60.50 | 61.89 |

**C=Control, T=Treatment (1% for 1.30hrs of EMS)**

**Table 3: Physiological Parameters**

| S.N. | Genotypes | RWC (%) | | Chlorophyll ($\mu gm/cm^2$) | | Photosynthesis rate ( $\mu mol/m^2sec$) | |
|---|---|---|---|---|---|---|---|
| | | C | T | C | T | C | T |
| 1 | HUW 510 | 56.07 | 55.23 | 48.40 | 47.35 | 16.80 | 15.78 |
| 2 | C 306 | 29.43 | 26.40 | 45.97 | 42.87 | 16.40 | 16.03 |
| 3 | Sonalika | 26.29 | 24.55 | 40.10 | 39.05 | 17.43 | 16.89 |
| 4 | HD 2135 | 55.19 | 53.25 | 40.55 | 39.02 | 17.67 | 16.83 |
| 5 | HD 2177 | 36.37 | 33.23 | 39.87 | 38.87 | 18.17 | 16.33 |
| 6 | VL 401 | 27.40 | 24.37 | 41.63 | 39.87 | 18.10 | 17.33 |
| 7 | K 9162 | 49.49 | 44.48 | 45.97 | 41.55 | 17.83 | 17.17 |
| 8 | RAJ 3765 | 60.69 | 61.73 | 49.25 | 50.88 | 22.57 | 23.57 |
| 9 | K 68 | 59.44 | 54.60 | 43.58 | 42.23 | 19.10 | 18.33 |
| 10 | K 7410 | 62.30 | 62.91 | 52.58 | 54.83 | 23.43 | 24.00 |

**C=Control, T=Treatment (1% for 1.30hrs of EMS)**

**Figure 1: Showing pre-harvest characters of wheat**

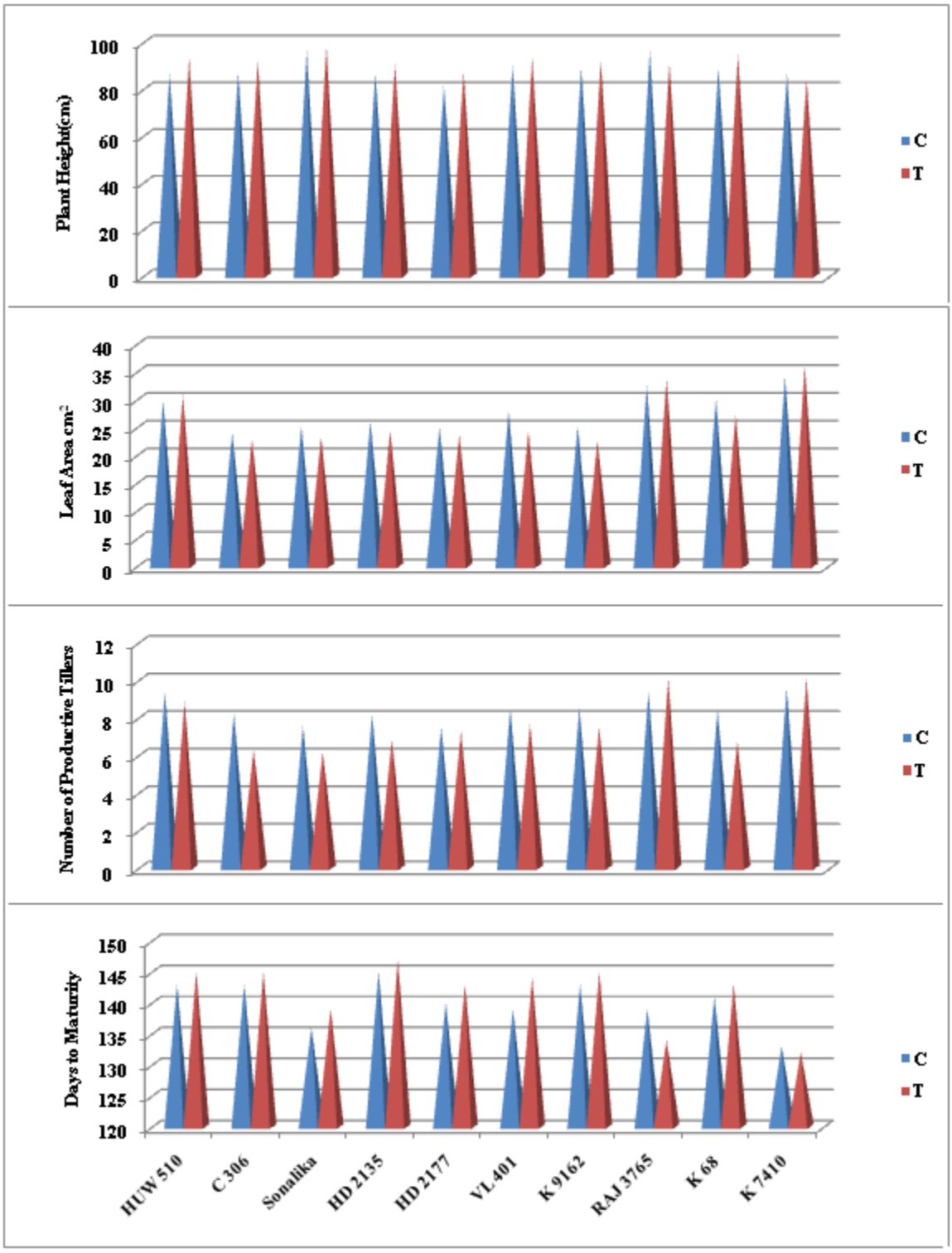

**Figure 2: Showing post-harvest characters of wheat**

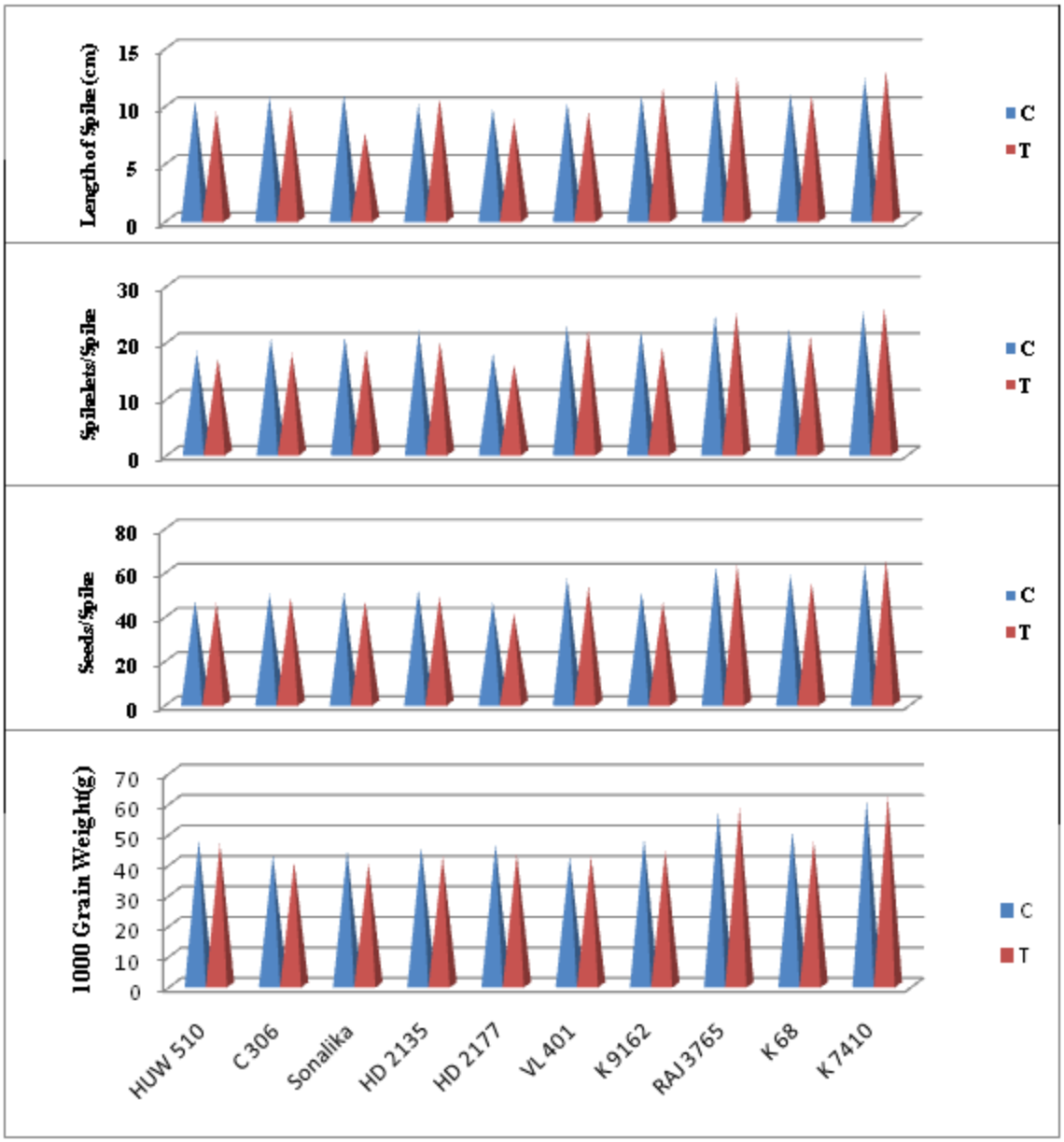

**Figure 3: Showing physiological parameters of wheat**