Indian Journal of Agricultural Sciences **92** (6): 700–4, June 2022/Article
https://doi.org/10.56093/ijas.v92i6.102508

Effect of endogenous gibberellic acid content on physiological and yield related traits in late sown wheat (*Triticum aestivum*)

SHIVANI NAGAR[1], V P SINGH[1]*, NEERA SINGH[1], RAJKUMAR DHAKAR[1] and AJAY ARORA[1]

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

Received: 16 July 2020; Accepted: 06 January 2022

ABSTRACT

Terminal heat stress is becoming a major factor in limiting wheat production with increasing evidence of heat stress in present scenario of climate change. Gibberellic acid is one of the major plant hormones playing crucial role in plant development from germination to seed development, but there is huge gap in knowledge about its role under heat stress. Present study was conducted in late sown wheat (2014–15) at ICAR-IARI, New Delhi to examine the inter-relationship between gibberellic acid (GA_3) level and physiological and yield related traits under heat stress in 40 wheat (*Trirtcum aestivum* L.) genotypes. Genotypes were categorized into three classes namely tolerant, intermediate and sensitive based on heat stress tolerance ability. The diversity in endogenous GA_3 concentration was found among the cultivars. The inverse relationship between GA_3 level in flag leaf and tolerance level of genotypes was found in this study through regression analysis ($P<0.05$). The path analysis showed that GA_3 had direct negative influence on test weight under heat stress conditions. Thus, investigations of mechanism of GA_3 influence on sink capacity has the potential to develop heat tolerant genotypes and increase wheat production under heat stress.

Keywords: Gibberellic acid, Heat susceptibility index, High temperature, Post anthesis, Wheat

Heat stress is second most important abiotic stress limiting crop production worldwide after drought. Introduction of rice–wheat cropping system led to delayed sowing of wheat (*Triticum aestivum* L.) in eastern Indo-Gangetic Plains and exposing wheat to heat stress at later stage of development (Joshi *et al.* 2007, Dwivedi *et al.* 2017). Terminal heat stress at the time of grain development is one of most prominent reason contributing to yield plateau and degradation of grain quality in wheat (Lobell *et al.* 2012). Heat stress affects flowering, pollen viability, availability and translocation of photosynthates to the developing kernel, starch synthesis and its deposition within the kernel, thus resulting in lower grain number, grain weight and grain quality (Gonzalez-Navarro *et al.* 2015). Plants reduce heat stress induced damages through adopting various morphological adjustments, transpirational cooling, delayed stress induced senescence, increased level of ROS scavenger and antioxidant enzymes, accumulation of osmolytes, increased abscisic acid and stress protein (HSFs, HSPs, dehydrins etc.) level, altered hormonal pool (Farooq *et al.* 2011).

[1]ICAR-Indian Agriculture Research Institute, New Delhi.
*Corresponding author email: drsinghvp@yahoo.com

Plants use endogenous hormones to couple their growth rate to changing environmental condition (Farooq *et al.* 2011). Murphy (2015) reported decreased cytokinin, auxin and gibberellic acid (GAs) and increased abscisic acid and ethylene content in heat stressed plants. GA plays important role in plant growth, development and physiological functions such as stimulating organ growth through enhancement of cell elongation and division, pollen fertility, stamen elongation, pollen tube growth, endosperm sink capacity and mobilization of starch to developing seed etc. (CoelhoFilho *et al.* 2014). It is well studied that endogenous GA_3 level decreases under heat stress (Vettakkorumakankav *et al.* 1999, Yamaguchi *et al.* 2008 and Ramírez *et al.* 2009) but very little is known about how endogenous GA_3 concentration varies among the different tolerant/susceptible genotypes under heat stress. Understanding the association between endogenous gibberellic acid level, and yield traits and heat stress tolerance ability in different genotypes is necessary for successful breeding programmes. In this study, we investigated general relationship of endogenous GA_3 level in leaves at post-anthesis stage with the heat stress tolerance level of 40 wheat genotypes.

MATERIALS AND METHODS

The 40 wheat genotypes were grown in the pots under field environmental conditions at the facility of Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi, India (28°41' North latitude and 77°13' East longitude, 228 m amsl) during *rabi* season of 2014–15. The details of 40 genotypes and their pedigree can be found in the study by Nagar *et al.* (2015). The earthen pots having size of 30 cm filled with 15 kg clay loam soil were used to grow the wheat genotypes. Farmyard manure in 3:1 ratio and N:P:K (dose of 60:60:60 kg/ha) fertilizers in the form of urea, single super phosphate and muriate of potash were mixed in the soil at the time of sowing. Remaining 60 kg N/ha was applied 25 days after sowing. The three replications (having five pots per replication) of each genotype were taken. The wheat plants were exposed to heat stress condition at the time of reproductive stage by late sowing (5th January, 2014) of wheat genotypes. Daily maximum atmospheric temperature was from 29–38°C after 15 days of 50% anthesis in late sown wheat which was nearly 4–7°C higher than the maximum atmospheric temperature of timely sown wheat (25–31°C).

Physiological observations, viz. Photosynthesis rate (Pn), Fv/Fm ratio, total chlorophyll, SPAD value, MSI were taken on fully expanded flag leaves after 15 days of anthesis and yield related observations like grain yield, test weight and HI were taken at the time of harvest. The endogenous GA_3 levels in genotypes were extracted from three uppermost fully opened leaves at 15 days after anthesis. Duration of each genotype was also recorded. The portable Infrared Gas Analyzer (IRGA), LI-6400XT Model (Li-COR Ltd., Lincoln, Nebraska, USA) was used to measure Pn and Fv/Fm with standard operating condition (relative humidity-50–60%, temperature 30–35°C, photosynthetic photon flux density-1200 μmol/m^2/s and CO_2 conc. 350–360 μmol/mol). The method of Sairam *et al.* (1997) was used to measure Membrane Stability Index (MSI). Test tube containing 10 ml of double distilled water with 100 mg of leaf material was placed in hot water bath. The initial conductivity (C_1) and final conductivity (C_2) on a conductivity bridge (Century, Water soil analysis kit, CMK 751) was recorded at 40°C and 100°C, respectively. MSI was calculated as:

$$\text{MSI} = [1 - (C_1/C_2)] \times 100$$

Total Chlorophyll was estimated in 0.05 g (w) of leaf sample in 10 ml (V) DMSO (dimethyl sulfoxide) by non-maceration method (Hiscox and Israelstam 1979). Absorbance was recorded at 645 (A_{645}) and 665 (A_{665}) nm then total chlorophyll was calculated using formula of Arnon (1949):

$$\text{Total chlorophyll} = (20.2 \times A_{645} + 8.02 \text{ x } A_{663}) \times V \times w/1000$$

SPAD chlorophyll meter reading were measured using SPAD from middle part of flag leaves using portable Minolta SPAD-502 chlorophyll meter (Minolta camera Co. Ltd., Osaka, Japan).

Gibberellic acid (GA_3) extraction was done using 5 g of three uppermost fully opened leaf. Sample was collected and frozen in liquid nitrogen and grounded into fine powder. Powder was kept in beaker filled with 30 ml methanol 70% (v/v) and stored at 4^{o}C until overnight. The extract was centrifuged at 5000 rpm for 15 min followed by filtering through Whatman filter and then methanol was evaporated under vacuum. The aqueous phase *p*H was adjusted to 2.5 with 1 N HCl then solution was partitioned with 50 ml diethyl ether (20 +20+10) thrice. Upper organic phase was collected in separate beaker after each washing. Collected sample was further passed through anhydrous sodium sulfate. After that, the diethyl ether was evaporated under vacuum and the dry residue containing hormones of GA_3 was dissolved in 2.0 ml of absolute methanol and stored in vial at 4°C. The chromatographic analysis was performed using Waters High Performance Liquid Chromatograph (HPLC) equipped with reversed phase column Crestpak C18 (150 mm × 4.6 mm i.d; 5 μm) maintained at constant temperature of 30±1°C. The mobile phase used was acetonitrile-water (30:70%, v/v), *p*H adjusted to 4.5 with orthophosphoric acid was delivered isocratically with flow rate of 1.0 ml/min. An injection volume of 10 μl was used for each analysis. The signal of the sample and standard was monitored at 208 nm for GA_3.

Heat Susceptibility Index (HSI) was computed as per Fisher and Maurer (1978)

$$\text{MSI} = (1\text{–Yh/Yn}) / (1\text{–Yg/Ya})/$$

where, Yh is mean yield of individual genotype under high temperature, Yn is mean yield of individual genotype under normal condition, Yg is mean yield of all genotypes under high temperature, Ya is mean yield of all genotypes under normal condition.

Statistical analysis: Linear regression analyses were used to determine the relationship between heat susceptibility index (HSI) and tolerance score with endogenous GA_3 level. Pearson's correlations were employed to determine the relationship between endogenous GA_3 level and other physiological traits and yield attributes under heat stress. The direct and indirect effect of physiological traits on heat tolerance/susceptibility was estimated by path analysis using M-Excel (Akintunde 2012). The test of significance for mean differences in endogenous GA level among the genotypes were evaluated using by one-way ANOVA and LSD tests ($P<0.05$) using 'r' statistical software. Nagar *et al.* (2015) categorized the 40 genotypes into three groups, viz. tolerant, intermediate and sensitive based on physiological. This categorization of genotypes with their tolerance score was used for further analysis.

RESULTS AND DISCUSSION

Temperatures above optimum range at reproductive phase have negative impact on wheat grain yield and grain quality. Hormonal homeostasis is also altered in stressed plants and role of hormones like abscisic acid, ethylene and cytokinin is well proven. Here, we have tried to explore the potential role of gibberellic acid under high temperature which still not studied widely. The categorization of 40 wheat genotypes for heat stress tolerance carried out by Nagar *et al.* (2015) is used in this study. Tolerance level of these wheat genotypes were decided by tolerance score which is computed using eight physiological traits (Nagar *et al.* 2015). Heat stress at reproductive stage brought changes in studied physiological traits like Pn, Fv/Fm, total chlorophyll, MSI, endogenous GA_3 content etc. and yield related traits like test weight grain yield. But magnitude of alteration in physiological and yield related traits under heat stress varied among genotypes. Many researchers in past have reported similar response of plants under heat stress (Farooq *et al.* 2011, Nagar *et al*. 2015, Dwivedi *et al.* 2017). The genotypes have been arranged in descending order of heat stress tolerance within and between the categories i.e. tolerant, intermediate and sensitive (Fig 1). Tolerance score of wheat genotypes given by Nagar *et al.* (2015) was primarily used for categorization rather than HSI because former was based on eight physiological traits while later was based on yield only. The results on variation in HSI of wheat genotypes under heat stress varied from 64.2 (HD 2932) to 130.52 (HUW 234) with the mean value of 101.22. The mean value of HSI under tolerant, intermediate and sensitive category was 87.63, 105.75 and 112.56, respectively. In this study, emphasis on variation in endogenous GA_3 content and heat susceptibility index among the tolerant/sensitive genotypes under heat stress was considered in detail. Variation of GA contents in different genotypes of crop plants under water stress is very rare (Yang *et al.* 2001, Xie *et al.* 2003). Alike, studies on variation in GA under heat stress is also uncommon. We found lower GA_3 content in leaves of tolerant cultivars, viz. LOK 1, HALNA, RAJ 3745, DBW 16, DBW 14 than mean value of 40 studied cultivars. HSI for yield showed marked differences among the genotypes under heat stress condition and obtained lower value for tolerant cultivars like LOK1,

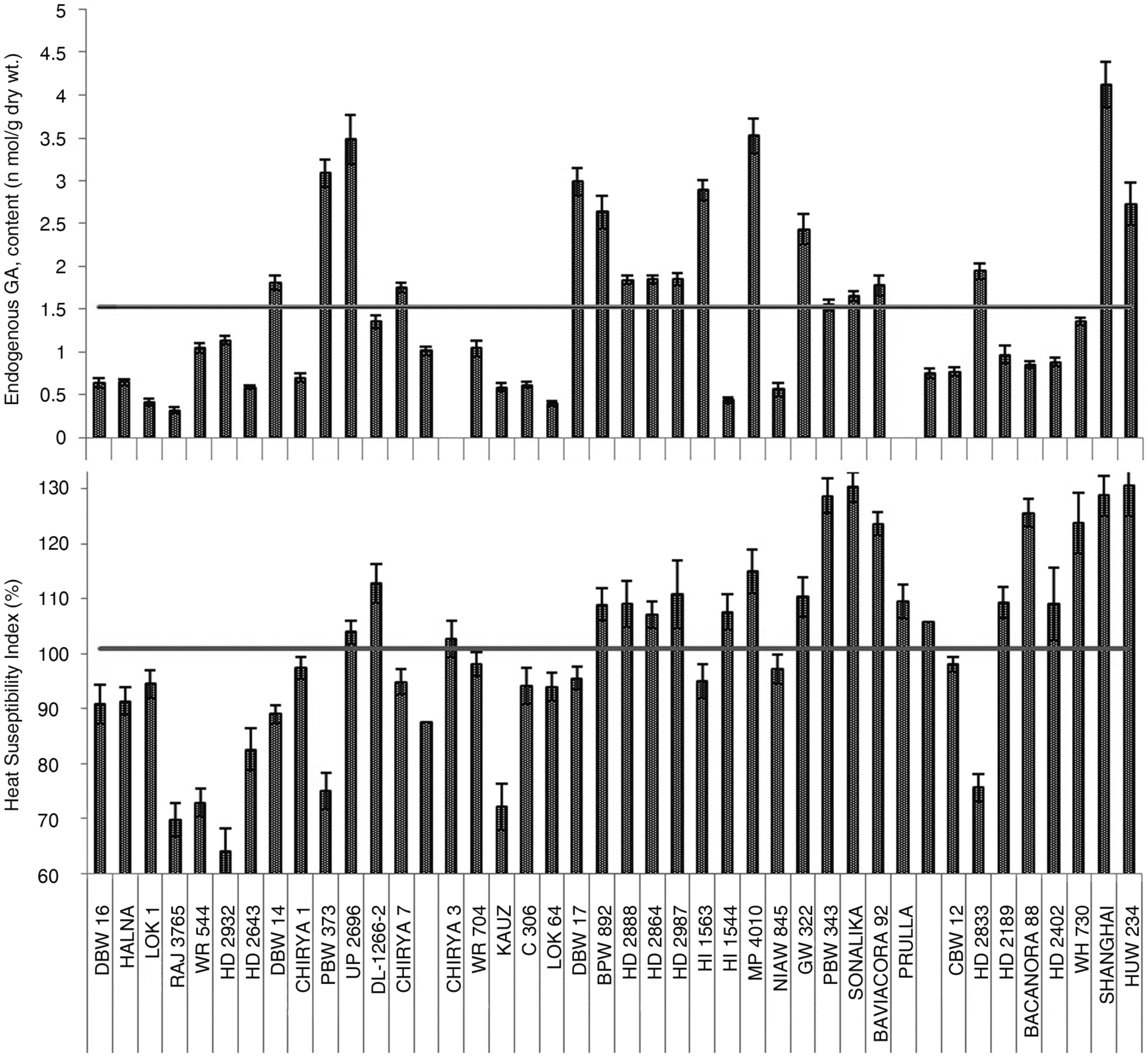

Fig 1 Variation in heat susceptibility index and endogenous GA_3 content (n mol/g dry wt.) in leaves of wheat cultivars at post anthesis stage under heat stress.

Table 1 Pearson's correlation coefficients between endogenous GA_3 content and physiological traits under heat stress.

Physiological trait	Correlation coefficient (r)
Total chlorophyll	0.13
Photosynthesis Rate	-0.27*
Fv/Fm	-0.17
MSI (%)	-0.30*
SPAD	-0.25
Grain yield (g/plant)	-0.19
Test wt (g)	-0.27*
HI (%)	-0.22
Duration	-0.10
HSI	0.32**

*significant at P<0.1, **significant at P<0.05

RAJ 3765, DBW 14, WR 544 etc. Dhyani *et al.* (2013) also reported lower HSI for yield in tolerant cultivars like RAJ 3765, DBW 14 and PBW 574 than sensitive cultivars like HS 240 and K-0-307. Thus, genotypes differ in ability to counteract heat stress and could be useful germplasm to develop wheat varieties under breeding program.

Endogenous GA_3 content in the leaves of wheat genotype at post anthesis stage under heat stress condition are presented in Fig 1. The concentration of GA_3 varied from 0.32 (RAJ 3765) to 4.12 (Shanghai). The mean value of GA_3 concentration in 40 wheat genotypes was 1.52. The mean value of endogenous GA_3 level in leaves of tolerant cultivars was 1.29 it was much lower than overall mean value with exceptionally high GA_3content in genotypes PBW 373 and UP 2696 in tolerant group. In sensitive category most genotypes had lower GA_3 content than mean value with exceptions of Purulla, Sanghai and HUW 234.Within the tolerant category, GA_3 level was lower in relatively tolerant cultivar than sensitive ones. In general, GA_3 level was higher in genotypes of intermediate category. However, low concentration of GA_3 was found in relatively tolerant genotypes within the intermediate category and same was the case with genotypes of sensitive category.

Exposure of plants to stressed environment accelerates the process of senescence which alters the hormonal pool of the plants. It leads to decrease in hormones like cytokinin, GA and auxin and increase in content of growth retarding and stress hormones like ABA and ethylene (Lehmann and Vlasov 1988). Decrease in endogenous GA content under stress condition is well known from previous studies (Kendall *et al.* 2011), which could be as an outcome of reduced GA biosynthesis resulting from the accelerating senescence of the organs in stressed environment (Yang *et al.* 2006, Xie *et al.* 2003). In present study, generally tolerant cultivars were able to maintain low level of endogenous gibberellic acid. Low level of GA_3 might be result of reduced GA biosynthesis due to accelerated senescence or it could be because of mobilization of GA from flag leaf to developing grain. GA_3 levels in leaves at 10 days post anthesis was non-significantly negatively related to grain yield under water stress condition (Xie *et al.* 2003). Similar results under heat stress condition were seen.

Relationship between endogenous GA_3 level and physiological traits under heat stress: From the correlation analysis (Table 1), it is evident that endogenous GA_3 content in leaves of wheat at post anthesis stage under heat stress condition is negatively related to Pn (r=-0.27), MSI (r=-0.30) and test weight (r=-0.27) which is statistically significant at P<0.1 while it is non-significantly correlated with total chlorophyll, Fv/Fm, SPAD, HI. Heat susceptibility index is positively related to endogenous GA_3 level which is statistically significant at P<0.05. This means that if wheat

Table 2 Estimated direct and indirect effect of physiological traits on post anthesis heat stress susceptibility in wheat cultivars

	Effect on Heat susceptibility								
	Total Chlorophyll	Pn	Fv/Fm	MSI	SPAD	GY	TW	HI	Duration
Direct effect	0.03	-0.29*	0.17	0.19	-0.27*	-0.15	-0.07	-0.37**	-0.20
Indirect effect via									
Total Chlorophyll	0.03	0.02	0.01	0.01	0.02	0.01	0.00	0.01	0.00
Pn	-0.15	-0.29	-0.22	-0.16	-0.23	-0.14	-0.06	-0.16	-0.01
Fv/Fm	0.07	0.12	0.17	0.07	0.09	0.07	0.06	0.08	0.03
MSI	0.06	0.11	0.08	0.19	0.10	0.14	0.02	0.12	0.00
SPAD	-0.18	-0.21	-0.15	-0.14	-0.27	-0.15	-0.03	-0.13	0.00
GY	-0.04	-0.07	-0.07	-0.11	-0.08	-0.15	-0.04	-0.12	0.00
TW	0.00	-0.01	-0.03	-0.01	-0.01	-0.02	-0.07	-0.03	-0.01
HI	-0.09	-0.20	-0.17	-0.24	-0.17	-0.29	-0.13	-0.37	-0.03
GA_3	0.01	-0.03	-0.02	-0.03	-0.03	-0.02	-0.03	-0.02	-0.01
Duration	0.01	-0.01	-0.03	0.00	0.00	0.01	-0.04	-0.02	-0.20
Total Indirect effect	-0.33	-0.29	-0.58	-0.62	-0.30	-0.39	-0.26	-0.26	-0.03
Correlation	-0.29	-0.58***	-0.41***	-0.43***	-0.58***	-0.54***	-0.33**	-0.63***	-0.23

plants are able to maintain lower endogenous GA_3 level in leaves at post anthesis stage under heat stress then they will be more tolerant to heat stress.

The direct and indirect effects of various physiological and yield-related traits on heat susceptibility index under heat stress conditions are shown in (Table 2). The results from path analysis reveals that susceptibility of plant to heat stress at post anthesis stage is negatively correlated to Pn, SPAD, grain yield and harvest index which are statistically significant at $P<0.001$. MSI and Fv/Fm have significant negative indirect effect and non-significant positive direct effect on susceptibility of plant to heat stress.

No study was found which depict the relationship of GA_3 content in leaves at post anthesis stage and sensitivity of wheat genotypes to heat stress. In present study, we found significant negative relation between the two through regression analysis. Path analysis revealed that endogenous GA_3 level in leaves at post anthesis stage does not directly influence heat stress susceptibility and also has very little indirect effect on other studied physiological parameters. We found that endogenous GA_3 content in leaves at post anthesis stage under heat stress had direct negative influence on test weight, which is a measure of sink capacity. Therefore, there is need to study more physiological parameters like antioxidants to see the influence of GA_3 content in leaves on stress susceptibility of plant under heat stress.

Overall it can be concluded that considerable variation among the genotypes is present for their GA_3 level in leaves at reproductive stage under heat stress. GA_3 had significant negative correlation with Pn, MSI and heat susceptibility index. It also had direct negative effect on sink capacity in terms of grain test weight under heat stress. Understanding the mechanism of gibberellic acid influence on sink capacity may have the potential to increase wheat production under heat stress. There is need of a detailed time course study about movement and role of GA during seed development under stressed condition to understand its importance in the process.

ACKNOWLEDGEMENTS

We thank Council for Scientific and Industrial Research, and ICAR-Indian Agricultural Research Institute for providing financial assistance for this research.

REFERENCES

Arnon D I. 1949. Copper enzymes in intact chloroplast, polyphenoxide in *Beta vulgaris*. *Plant Physiology* **24**: 1–15.

Coelho Filho M A, Colebrook E H, Lloyd D P, Webster C P, Mooney S J, Phillips A L, Hedden P and Whalley W R. 2013. The involvement of gibberellin signalling in the effect of soil resistance to root penetration on leaf elongation and tiller number in wheat. *Plant and soil* **371**(1–2): 81–9

Dhyani K, Ansari M W, Rao Y R, Verma, R S, Shukla A and Tuteja N. 2013. Comparative physiological response of wheat genotypes under terminal heat stress. *Plant signalling behaviour* **8**(6): 245–64.

Dwivedi S K, Basu S, Kumar S, Kumar G, Prakash V, Kumar S, Mishra J S, Bhatt B P, Malviya N, Singh G P and Arora A. 2017. Heat stress induced impairment of starch mobilisation regulates pollen viability and grain yield in wheat: Study in Eastern Indo–Gangetic Plains. *Field Crops Research* **206**: 106–14.

Farooq M, Bramley H, Palta J A and Siddique K H. 2011. Heat stress in wheat during reproductive and grain-filling phases. *Critical Reviews in Plant Sciences* **30**(6): 491–507.

Gonzalez-Navarro O E, Griffithsb S, Moleroa G, Reynolds M P and Slaferc G A. 2015. Dynamics of floret development determining differences in spike fertility in an elite population of wheat. *Field Crops Research* **172**: 21–31

Hiscox J D and Israelstam G F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany* **57**: 1332–34.

Joshi A K, Chand R, Arun B, Singh R P and Ortiz R. 2007. Breeding crops for reduced-tillage management in the intensive, rice–wheat systems of South Asia. *Euphytica* **153**(1-2): 135–51.

Kendall S L, Hellwege A, Marriot P, Whalley C, Graham I A and Penfield S. 2011. Induction of dormancy in Arabidopsis summer annuals requires parallel regulation of DOG1 and hormone metabolism by low temperature and CBF transcription factors. *The Plant Cell* **23**(7): 2568–80.

Lehmann H and Vlasov P. 1988. Plant growth and stress- the enzyme hydrolysis of abscisic acid conjugate. *Journal of Plant Physiology* **132**: 98–101.

Lobell D B, Sibley A and Ortiz-Monasterio J I. 2012. Extreme heat effects on wheat senescence in India. *Nature Climate Change* 2: 186–89.

Murphy A. 2015. Hormone crosstalk in plants. *Journal of Experimental Botany* **66**(16): 4853–54.

Nagar S, Singh V P, Arora A, Dhakar R and Ramakrishnan S. 2015. Assessment of terminal heat tolerance ability of wheat genotypes based on physiological traits using multivariate analysis. *Acta Physiologiae Plantarum* **37**(12): 1–9.

Sairam R K, Deshmukh P S and Shukla D S. 1997. Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. *Journal of Agronomy and Crop Science* **178**(3): 171–78.

Toker C and Cagirgan M I. 2004.The use of phenotypic correlation and factor analysis in determining characters for grain yield selection in chickpea (*Cicer arietinum* L.). *Heriditas* **140**(3): 226–28.

Vettakkorumakankav N N, Falk D, Saxena P and Fletcher R A. 1999. A crucial role for gibberellins in stress protection of plants. *Plant Cell Physiology* **40**: 542–48.

Xie Z, Jiang D, Cao W, Dai T and Jing Q. 2003. Relationships of endogenous plant hormones to accumulation of grain protein and starch in winter wheat under different post-anthesis soil water statuses. *Plant growth regulation* **41**(2):117–27.

Yamaguchi S. 2008. Gibberellin metabolism and its regulation. *Annual Review of Plant Biology* **59**: 225–51.

Yang J, Zhang J, Liu K, Wang Z and Liu L. 2006. Abscisic acid and ethylene interact in wheat grains in response to soil drying during grain filling. *New Phytologist* **171**(2): 293–303.

Yang J, Zhang J, Wang Z, Zhu Q and Wang W. 2001. Hormonal changes in the grains of rice subjected to water stress during grain filling. *Plant Physiology* **127**(1): 315–23.