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Stay-green trait ameliorates combined heat and drought stress in wheat

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ABSTRACT

The present study was carried out during *rabi* 2017-18 at ICAR-Indian Agricultural Research Institute, New Delhi to investigate the effect of stay-green trait on grain yield in wheat under combined heat and drought stress. Two high stay green (GCP 6 and GCP 33) and two low stay green (GCP 23 and GCP 30) Recombinant Inbred Lines (RILs) of wheat along with their parents (HI1500/DBW43) were grown in field conditions under timely sown with irrigation (control) and without irrigation (drought stress), and late sown with irrigation (heat stress) and without irrigation (combined heat and drought stress). High stay-green RILs showed the reduced levels of abscisic acid and ethylene production during anthesis stage under heat, drought and combined stress conditions as compared to low stay green RILs. Furthermore, there was significantly better yield was observed in high stay-green RILs as compared to low stay-green RILs. Thus our study concludes that stay-green traits improve the yield in wheat under combined heat and drought stress condition by delaying the senescence through reduced levels of abscisic acid and ethylene.

Keywords: Combined stress, Flag leaves, Stay-green, Wheat, Yield

Wheat is one of the most important food crops that is cultivated on approximately 223.67 million ha of land and contributes 735.3 million tons in terms of global production (USDA 2017). Heat and drought are the two most important abiotic stresses which limit the wheat production and both stresses at same time are more lethal. Leaf stay-green is a trait in which leaves retain green tissue from flowering to physiological maturity instead of senescing and that is considered as an important for grain filling (Kamal *et al*. 2019). Previous studies showed that the senescence is regulated by hormones like abscisic acid (ABA), ethylene, and cytokinin. Djanaguiraman and Prasad (2010) demonstrated that there is reduction in photosynthesis rate and sucrose content and also inhibition of key enzymatic antioxidants in soybean after heat treatment due to enhanced ethylene production that ultimately triggers premature leaf senescence. A high ethylene level in developing seeds leads to poor grain filling due to negative correlation with starch metabolism-related enzyme activities (Zhang *et al*. 2009). Furthermore it is well documented that in response to abiotic stresses such as drought, high salinity, and low temperatures, ABA level promotes leaf senescence (Abhinandan *et al*. 2018).

Although the role of ABA and ethylene under high temperature and drought stress are well documented but still their role under combined stress is unclear regarding maintenance of stay-green trait and yield. So the purpose of this study was to investigate how the stay-green RILs delay the process of senescence by regulating ABA and ethylene levels at anthesis and post anthesis stages in the flag leaves of wheat under combined stress of drought and heat.

MATERIALS AND METHODS

Experimental material, design and treatment: The field experiment consisting of two high stay-green (GCP 6 and GCP 33) and two low stay-green (GCP 23 and GCP 30) Recombinant Inbred Lines (RILs) along with their parents HI1500/DBW43 was carried at ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India in *rabi* during 2017–18. The contrasting stay-green RILs used here were screened in our lab on the basis of total chlorophyll content, photosynthesis rate, leaf area duration and yield attributes (Unpublished data). DBW43 and HI1500 are moderately tolerant to drought and heat stress. Material for the study comprised of 6 (RILs + 2 Parents) genotypes of wheat, which were evaluated under timely sowing irrigated (Control) and without irrigation (drought) along with late sowing with irrigation (heat stress) and without irrigation (combined heat & drought stress) during *rabi* 2017–18 by following randomized block design with three replications.

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The maximum temperature recorded at the time of anthesis under control (22.8 to 25.0^{0}C), drought (25 to 27.5^{0}C), heat (29.2 to 36.8^{0}C) and combined stress (29.2 to 33.2^{0}C) conditions indicated that our late sown experiment faced high temperature stress at the anthesis stage.

Soil moisture measurements: The soil moisture content was measured by gravimetric method and further correlated with neutron moisture meter readings. Mean plant available water content in the root zone at anthesis stage was 23.06–24.51% for control, 13.72–12.82% for drought, 17.29–16.33% for heat, and 7.96–7.35% for the combined stress conditions.

Plant physiological traits: Plant stress indicator traits like relative water content (RWC) and membrane stability index (MSI) were measured in flag leaf at anthesis stage by using the methods suggested by Barrs and Weatherly (1962) and Premachandra *et al*. (1990), respectively. Total chlorophyll content (mg g^{-1} DW) was measured in flag leaf from anthesis (A) to A+5 days under control, drought, heat and combined stress conditions by using the method described by Hiscox and Israelstam (1979). The photosynthetic rate (µmoles CO_2 m^{-2} s^{-1}) of flag leaf was measured at anthesis stage using the portable Infrared Gas Analyser (IRGA), LI-6400XT Model. Leaf area index was also measured per meter square basis. Leaf area index was measured at anthesis (A) and A+5 days under control, drought, heat and combined stress conditions.

Ethylene emission rate and abscisic acid (ABA) estimation: Ethylene emission rate (nmol/g DW/h) and abscisic acid content (µg/g DW) was estimated in flag leaves through gas chromatography (Beltrano *et al*. 1994) and High Performance Liquid Chromatography (Zeevaart *et al.* 1980) respectively.

Yield components: Grain yield (g/m^{-2}) and 1000-grain weight (g) were estimated for control, drought, heat and combined stress conditions.

Total RNA isolation and cDNA synthesis: Total RNA was extracted from flag leaf tissues by using TRIzol® reagent (Invitrogen, USA). Complementary DNA (cDNA) was synthesized from isolated RNA by using the cDNA Synthesis Superscript® III First- Strand Synthesis Kit (Invitrogen, USA). Resulting cDNA was stored at -20^{0}C and employed as a template for Real-Time quantitative PCR (RT- PCR) reactions.

Primer designing and Real-Time quantitative PCR: Real-Time quantitative PCR primers (Supplementary Table 1) were designed using OligoAnalyzer 3.1. Real time quantitative PCR reaction was performed using a Step One PlusTM real-time detection system (Applied biosystems).

Statistical analysis: Statistical analysis was done with ANOVA and Duncan' test at significant difference (P<0.05) by using SPSS 16.0 (SPSS Inc., Chicago, IL, USA)**.**

RESULTS AND DISCUSSION

There was a significant difference in relative water content (RWC), membrane stability index (MSI) and photosynthesis rate (Pn rate) between control, drought, heat and combined stress conditions at anthesis stage. Furthermore it was observed that high stay-green RILs retained higher total chlorophyll content, leaf area index and yield as compared to low stay-green RILs (Table 1).

Ethylene emission sharply increases in the flag leaves under combined stress: We observed the mean % change in ethylene emission rate under drought, heat and combined stress at anthesis stage was 45.04%, 85.40% and 158.25%, respectively as comparison to control condition. Under all the stress conditions high stay-green RILs (GCP 6 and GCP 33) showed significantly less ethylene emission rate than low stay-green RILs (GCP 23 and GCP 30) at the time of anthesis and post-anthesis stages (Fig 1). Our study revealed that under combined stress, there was significantly higher rate of ethylene emission by flag leaves at the time of anthesis as compared to individual heat and drought stress. Previous studies indicated that increased ethylene emission rate shorten the grain filling period by triggering premature senescence and hastening the maturity that leads to decreased 1000 kernel weight in rice, wheat and maize (Yang *et al.* 2006). The same we have observed in our study, viz. low stay-green RILs compromised yield (g/m^2), whereas high stay-green RILs stabilizes/maintained the same under combined stress condition (Table 1).

TaACS1 and TaACO2 genes expression repressed in stay-green genotypes under combined stress: ACC synthase and ACC oxidase are two key enzymes for ethylene biosynthesis that are known to be encoded by multigene families in seed plants. The gene expression of *ACS1* and *ACO2* was found to be significantly higher under drought, heat and combined stress in all the genotypes (4RILs + 2 parents). The high stay-green RILs showed significantly lower expression of *TaACS1* and *TaACO2* under drought, heat and combined stress as compared to low stay-green RILs. Previous studies revealed that there was induced synthesis of ACS under stress condition, which subsequently

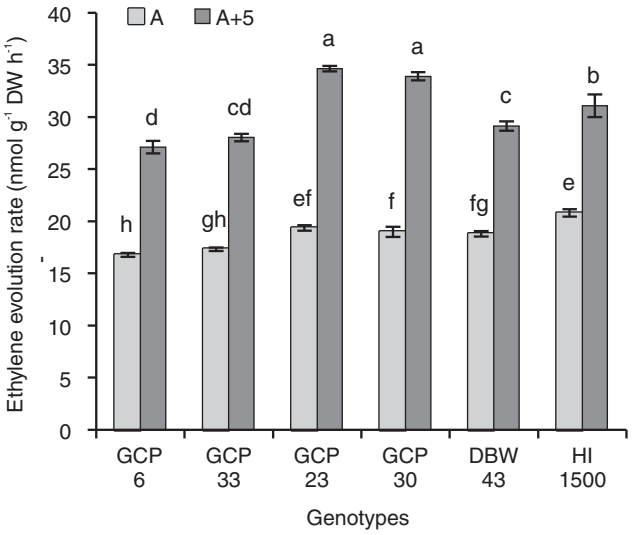

Fig 1 Ethylene evolution in the flag leaves of wheat genotypes at anthesis and post-anthesis stages under combined stress (A to 5 DAA) condition.

Table 1 Mean values of RWC, MSI, photosynthesis rate, total chlorophyll, leaf area index and yield for contrasting RILs of wheat for cropping season 2017-18 under control [C], drought [D], heat [H] and combined [HD] stress conditions.

Treatment	Genotypes	RWC (%) at anthesis	MSI at anthesis	Pn rate (µmole CO_2 /m^2/s) at anthesis	Total chlorophyll (mg g^{-1} DW)		Leaf area index		Yield (g m^{-2})
					Anthesis	Anthesis+5	Anthesis	Anthesis+5	
C	GCP 6	87.93±0.92	86.49±1.06	27.26±1.55	21.19±0.80	19.11±0.08	3.58±0.16	3.45±0.16	431±4.18
	GCP 33	87.86±0.66	86.10±1.03	27.07±0.74	20.84±0.43	20.17±0.85	3.15±0.06	2.69±0.23	420.93±6.36
	GCP 23	83.04±1.46	81.23±0.56	21.44±0.79	18.02±0.56	17.35±0.56	2.53±0.14	2.52±0.01	360.63±5.54
	GCP 30	83.87±1.16	82.47±1.30	21.60±0.52	18.54±1.08	17.56±0.27	2.58±0.23	2.40±0.11	381.17±2.72
D	GCP 6	74.58±2.18	76.25±0.52	22.88±1.19	14.72±0.52	13.30±0.25	3.08±0.22	2.74±0.13	360.83±9.71
	GCP 33	73.41±0.81	75.50±0.66	22.33±0.96	14.07±0.34	13.04±0.39	2.63±0.06	2.18±0.30	350.23±15.30
	GCP 23	64.83±5.06	69.88±1.07	16.69±1.28	12.00±0.47	10.97±0.26	1.94±0.10	1.88±0.09	270.90±12.99
	GCP 30	65.24±0.74	72.79±0.86	17.06±1.33	12.42±0.16	11.74±0.61	1.96±0.34	1.98±0.12	291.20±10.81
H	GCP 6	78.05±4.81	76.74±1.07	19.27±.01	11.39±0.19	8.39±0.28	1.84±0.17	1.64±0.11	295.40±15.31
	GCP 33	76.55±1.65	76.44±1.09	18.33±0.32	10.66±0.40	8.35±0.46	1.74±0.10	1.46±0.15	290.90±15.77
	GCP 23	64.93±0.59	70.41±1.58	14.31±1.27	8.70±0.17	6.50±0.09	0.64±0.04	0.54±0.05	210.17±15.34
	GCP 30	66.67±3.95	72.63±0.69	14.68±0.42	9.23±0.38	6.94±0.18	0.73±0.10	0.60±0.02	222.10±11.93
HD	GCP 6	71.73±1.04	71.10±2.15	15.05±0.99	8.46±0.24	6.04±0.23	0.86±0.04	0.71±0.23	215.37±18.34
	GCP 33	71.11±4.16	69.99±2.15	14.54±0.49	8.41±0.07	5.99±0.08	0.69±0.01	0.57±0.00	212.63±16.91
	GCP 23	53.94±2.73	59.37±3.63	10.08±1.21	6.44±0.24	3.76±0.07	0.62±0.01	0.47±0.03	140.77±18.70
	GCP 30	57.93±2.32	59.69±1.37	11.09±1.20	6.66±0.29	4.33±0.24	0.64±0.02	0.50±0.00	144.57±13.45

triggered rapid accumulation of ACC and a marked increase in ethylene production (Achard *et al.* 2006). Altogether our gene expression data are consistent with previous studies that the increased ethylene emission rate at the time of anthesis and post anthesis stage under combined stress is due to higher expression of key ethylene biosynthesis genes *TaACS1 and TaACO2.*

Stay-green RILs restrict abscisic acid production in the flag leaves under combined stress condition: We observed that as compared to control condition the mean % change in abscisic acid content in drought, heat and combined stress at anthesis stage was 348.75%, 363.40% and 575.66%, respectively. Under all the treatment conditions high stay-green RILs, GCP 6 and GCP 33 showed significantly less abscisic acid accumulation than low stay-green RILs, GCP 23 and GCP 30 at the time of anthesis and subsequent stages in the flag leaves (Fig 2). Previous studies also reported that ABA can induce leaf yellowing and serves as an indicator of leaf senescence (Fang *et al*. 2008). He *et al.* (2005) reported that stay-green cultivar of maize (P3845) blocked the translocation of ABA from roots to shoot and thus delaying the senescence process. Altogether our high stay-green RILs showed delayed senescence due to low ABA accumulation in flag leaf at anthesis and post anthesis stage as compared to low stay-green RILs.

TaNCED3 and TaAAO3 genes expression repressed in stay-green genotypes under combined stress: 9-*cis*-epoxycarotenoid dioxygenase (NCED) and ABA aldehyde oxidase (AAO) are considered as important enzymes in ABA biosynthesis pathway and encoded by multigene families in plants. We found that the gene expression of *TaNCED3 and TaAAO3,* significantly increased under drought, heat and combined stress over control in all the genotypes (4RILs + 2 parents). The high stay-green RILs showed significantly lower expression of *TaNCED3* and *TaAAO3* under drought, heat and combined stress as compared to low stay-green RILs. The previous studies also documented that ABA biosynthesis genes are up-regulated in drought stress (Seiler *et al.* 2011).

NCED3 expression has been found to be induced under

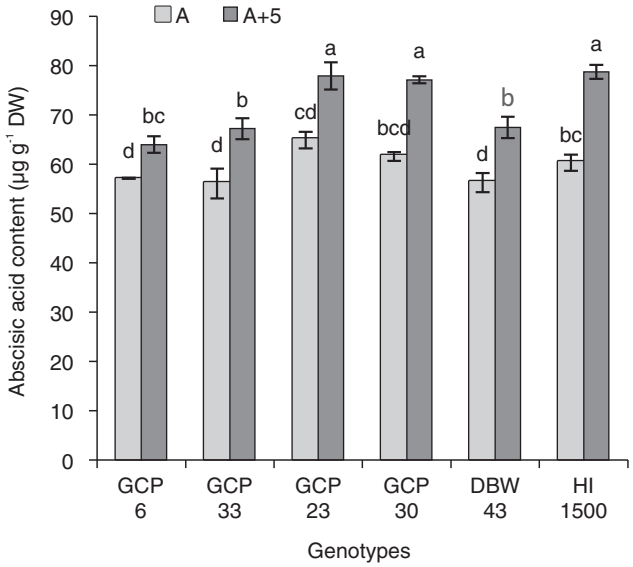

Fig 2 ABA content in the flag leaves of wheat genotypes at anthesis and post-anthesis stages under combined stress (A to 5 DAA) condition.

Table 2 Pearson correlation coefficients of ethylene emission rate and ABA content with stay-green traits (total chlorophyll, photosynthesis rate, LAI) and yield attributes in wheat under combined stress condition

Particulars		Pn (µmole CO_2 /m^2 /s)	Total Chls (mg g^{-1} DW)		LAI		Ethylene (n mol/g DW)		ABA (µg/g DW)		Yield (g/m^2)	TW (g)
	Stage	A	A	A+5	A	A+5	A	A+5	A	A+5		
Ethylene (n mol/g DW)	A	-0.29	-0.35	-0.53*	-0.36	-0.38	1	0.33	0.32	0.35	-0.386	-0.41
	A+5	-0.82**	-0.66**	-0.84**	-0.64**	-0.72**	0.33	1	0.87**	0.815**	-0.69**	-0.77**
ABA (µg/g DW)	A	-0.81**	-0.63**	-0.79**	-0.73**	-0.84**	0.32	0.87**	1	0.778**	-0.59**	-0.80**
	A+5	-0.66**	-0.61**	-0.83**	-0.45	-0.67**	0.35	0.81**	0.78**	1	-0.50*	-0.63**

** significance at 1 % level of significance, *significant at 5% level of significance. Total_Chls = Total chlorophyll content, Pn = Photosynthesis rate, LAI = Leaf Area Index, Yield = Yield/m^2 and TW = Test weight.

drought stress in maize, Arabidopsis, avocado, tomato, cowpea and bean (Ali *et al*. 2020). Zdunek-Zastocka (2008) revealed that under salt stress condition, *AAO3* expression increased in pea leaves. Moreover, it has been also reported that *Arabidopsis* mutants with deficiencies in ABA biosynthesis exhibit altered or delayed senescence (Passioura 2007). Our result is consistent with previous studies, high stay-green RILs delay senescence by minimizing expression of ABA biosynthesis genes.

TaABA8' OH1 gene highly expressed in stay-green genotypes under combined stress: ABA8' hydroxylase (ABA8'OH) degrades active ABA into 8'hydroxy-ABA and it is considered as a major route of ABA degradation. We found that the expression of *TaABA 8' OH1* increased significantly under drought, heat and combined stress over control in all the genotypes (4RILs + 2 parents). The high stay-green RILs showed significantly higher expression of *TaABA*8' *OH1* under drought, heat and combined stress as compared to low stay-green RILs. Seiler *et al*. (2011) reported that when ABA level increased in flag leaves of barley during drought stress, expression of *ABA8'OH1* prominently increased to adjust the higher ABA level. Our results are consistent with previous reports that high stay-green RILs lower the ABA content by higher expression of *TaABA*8'*OH1* under combined stress, while low stay-green RILs maintained higher ABA level.

Correlation of ABA and ethylene with stay-green traits and yield attributes under combined stress: We found that ABA content in the flag leaf at the anthesis stage was negatively and significantly correlated with total chlorophyll content, photosynthesis rate and leaf area index (Table 2). Additionally, ethylene emission rate from flag leaf at the anthesis stage was also negatively correlated with total chlorophyll content, photosynthesis rate and leaf area index but this correlation was not significant (Table 2). Furthermore, both ABA content and ethylene emission rate from flag leaf at the post anthesis stage (A+5) was found negatively and significantly correlated with total chlorophyll, leaf area index, yield and test weight. Interestingly, we also observed that the ABA content was positively correlated with ethylene evolution rate at anthesis and post-anthesis, although only at post anthesis stage this correlation was significant.

Earlier studies reported that photosynthesis rate and leaf area increased by low ethylene emission rate and reduced by higher ethylene emission rate (Khan 2005). Our study was highly consistent with the previous study that the high ethylene emission generally induces senescence and chlorophyll degradation (Iqbal *et al*. 2017). Moreover, low photosynthetic efficiency in drought stress reduces the grain-filling rate in wheat (Farooq *et al*. 2014). This indicates that ethylene is responsible for decrease in photosynthetic rate under combined stress conditions. Altogether our results showed that ABA elicits senescence probably by increasing ethylene synthesis, which further promote senescence.

Based on our experimental finding, we conclude stay-green can be an important factor to stabilize the yield under combined heat and drought stress conditions. Our study showed that the high stay-green RILs (GCP 6 and GCP 33) were found to stabilize the yield through delaying the senescence process by minimizing ABA and ethylene hormone production at the level of both biosynthesis and degradation. Further, there is a research scope to decipher the further underlying relationship between hormonal signaling and stay-green traits which can be important findings in crop improvement under changing climate scenario.

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REFERENCES

Abhinandan K, Skori L, Stanic M, Hickerson N, Jamshed M and Samuel M A. 2018. Abiotic stress signaling in wheat–an inclusive overview of hormonal interactions during abiotic stress responses in wheat. *Frontiers in Plant Science* **9**: 734.

Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten D, Peng J and Harberd N P. 2006. Integration of plant responses to environmentally activated phytohormonal signals. *Science* **311**(5757): 91–94.

Ali S, Hayat K, Iqbal A and Xie L. 2020. Implications of abscisic acid in the drought stress tolerance of plants. *Agronomy* **10**(9): 1323.

Barrs H D and Weatherley P E. 1962. A re-examination of the

relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences* **15**(3): 413–28.

Beltrano J, Carbone A, Montaldi E R and Guiamet J J. 1994. Ethylene as promoter of wheat grain maturation and ear senescence. *Plant Growth Regulation* **15**(2): 107–12.

Djanaguiraman M and Prasad P V. 2010. Ethylene production under high temperature stress causes premature leaf senescence in soybean. *Functional Plant Biology* **37**(11): 1071–84.

Fang J, Chai C, Qian Q, Li C, Tang J, Sun L, Huang Z, Guo X, Sun C, Liu M and Zhang Y. 2008. Mutations of genes in synthesis of the carotenoid precursors of ABA lead to pre-harvest sprouting and photo-oxidation in rice. *Plant Journal* **54**(2): 177–89.

Farooq M, Hussain M and Siddique K H. 2014. Drought stress in wheat during flowering and grain-filling periods. *Critical Reviews in Plant Sciences* **33**(4): 331–49.

He P, Osaki M, Takebe M, Shinano T and Wasaki J. 2005. Endogenous hormones and expression of senescence-related genes in different senescent types of maize. *Journal of Experimental Botany* **56**(414): 1117–28.

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**(12): 1332–34.

Iqbal N, Khan N A, Ferrante A, Trivellini A, Francini A and Khan M I R. 2017. Ethylene role in plant growth, development and senescence: interaction with other phytohormones. *Frontiers in Plant Science* **8**: 475.

Kamal N M, Gorafi Y S A, Abdelrahman M, Abdellatef E and Tsujimoto H. 2019. Stay-green trait: A prospective approach for yield potential, and drought and heat stress adaptation in globally important cereals. *International Journal of Molecular Sciences* **20**(23): 5837.

Khan N A. 2005. The influence of exogenous ethylene on growth and photosynthesis of mustard (*Brassica juncea*) following defoliation. *Scientia Horticulturae* **105**(4): 499–505.

Passioura J. 2007. The drought environment: physical, biological and agricultural perspectives. *Journal of Experimental Botany* **58**(2): 113–17.

Premachandra G S, Saneoka H and Ogata S. 1990. Cell membrane stability, an indicator of drought tolerance as affected by applied nitrogen in soybean. *Journal of Agricultural Science Cambridge* **115**: 63–66.

Seiler C, Harshavardhan V T, Rajesh K, Reddy P S, Strickert M, Rolletschek H, Scholz U, Wobus U and Sreenivasulu N. 2011. ABA biosynthesis and degradation contributing to ABA homeostasis during barley seed development under control and terminal drought-stress conditions. *Journal of Experimental Botany* **62**(8): 2615–32.

United States Department of Agriculture. 2017. World agricultural production. Washington, DC, USA.

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.

Zdunek-Zastocka E. 2008. Molecular cloning, characterization and expression analysis of three aldehyde oxidase genes from *Pisum sativum* L. *Plant Physiology and Biochemistry* **46**(1): 19–28.

Zeevaart J A. 1980. Changes in the levels of abscisic acid and its metabolites in excised leaf blades of *Xanthium strumarium* during and after water stress. *Plant Physiology* **66**(4): 672–78.

Zhang H, Tan G, Yang L, Yang J, Zhang J and Zhao B. 2009. Hormones in the grains and roots in relation to post-anthesis development of inferior and superior spikelets in *japonica/indica* hybrid rice. *Plant Physiology and Biochemistry* **47**(3): 195–204.