LEAF SENESCENCE AND CARBON ISOTOPE DISCRIMINATION IN DURUM WHEAT (*Triticum durum* Desf.) UNDER SEVERE DROUGHT CONDITIONS

M. Hafsi[1,✉], J. Akhter[2], P. Monneveux[3]

[1]*Université Ferhat Abbas, Sétif, Département d'Agronomie, Faculté des Sciences de la nature et de la vie ,Algeria*

[2] *Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan*

[3]*International Maize and Wheat Improvement Center (CIMMYT), Mexico City, Mexico*

✉Corresponding author: *hafsimiloud@yahoo.com*

Résumé*:* Les relations entre la senescence foliaire, la discrimination isotopique du carbone et la performance du rendement, sont examinées chez le Blé dur (Triticum durum Desf.), au niveau des hautes plaines sétifiennes. Dix variétés de Blé dur à haut potentiel ont été conduits durant deux saisons agricoles successives caractérisées par une faible pluviométrie (217 et 162 mm, respectivement), des températures gélives au stade épiaison et un stress thermique en fin de cycle. La sénescence a été évaluée par le traitement numérique de l'image (NIA). La discrimination isotopique du carbone de la feuille drapeau a été analysée pour les différentes variétés aux stades anthèse et maturité du grain respectivement. La sénescence a été corrélée significativement et négativement au rendement grain pour la première saison mais pas pour la deuxième. Il n'y a pas eu de relations significatives entre Δ et le rendement pour les deux saisons. L'absence d'association entre Δ (la discrimination isotopique du carbone) et le rendement grain est probablement dû à une forte contribution des assimilats au rendement au stade pré-anthèse avec une limitation du puits (fertilité de l'épi).

Mots clés : discrimination isotopique du carbone, sécheresse, sénescence, blé dur (*Triticum durum* Desf.)

Abstract: The relationships between leaf senescence, carbon isotope discrimination and yield performance were examined in durum wheat (*Triticum durum* Desf.), in the high plains of Setif, eastern Algeria. Ten CIMMYT high-yielding cultivars were grown during two cropping seasons characterized by low rainfall (217 and 162 mm, respectively), freezing temperatures at heading stage and terminal heat stress. Senescence was assessed using numerical image analysis (NIA). Carbon isotope discrimination was analyzed in flag leaves at anthesis and grain at maturity. Senescence was significantly negatively correlated to grain yield in season 1, but not in season 2. There was no relationship between Δ and grain yield in both seasons. The absence of association between Δ and grain yield is likely to be due a strong contribution of pre-anthesis assimilates to yield together with a sink limitation of yield.

Key-words: carbon isotope discrimination, drought, senescence, durum wheat (*Triticum durum* Desf.)

Introduction

Crop productivity in wheat is mainly related to photosynthetic activity, total leaf area, and leaf green area duration. Under drought conditions, photosynthetic activity and duration are strongly limited by reduction of stomatal conductance (Morgan et al., 1993) and premature leaf senescence (Pajević et al., 1999), respectively. Slow rates of senescence were found to be associated to higher yield in wheat by Rawson et al. (1983) and Ellen (1987). In some studies, however, quick senescence increased kernel weight and grain yield of wheat (Yang et al., 2001). Senescence is coupled with remobilization (Yang et al., 2001) that in some cases highly contributes to maintain grain yield (Gebbing and Schnyder, 1999). Senescence rate in wheat is particularly sensitive to water stress (Guo et al., 1998; Mi et al., 1999) and heat stress (Paulsen, 1994). Genetic variation for this trait has been reported in durum wheat (*Triticum durum* DESF.) (Hafsi et al., 2000). Evaluation of senescence still remains difficult. Progression of senescence from the tip to the base of the blade is heterogeneous and visual evaluation of the percentage of leaf affected by senescence consequently inaccurate. Dymond and Trotter (1997) and Clarke (1997) used digital cameras to assess crop greenness. Adamsen et al. (1999) developed this method to measure the senescence of wheat canopies. Hafsi et al. (2000) modified the technique to evaluate senescence of wheat leaves.

Under terminal (post-anthesis) water stress, wheat yield is associated with the capacity of the plant to maintain CO_2 assimilation (Morgan et al., 1993). Under field conditions a wide range of environmental factors and their interactions make difficult to detect genetic variation for this trait. Isotopic methods represent an alternative to gas exchange measurements. In C_3 plants, carbon isotope discrimination is a good long-term indicator of stomatal conductance and transpiration efficiency (Farquhar et al., 1989). In Mediterranean environments, higher yield is generally associated to high grain Δ (Araus et al., 1998; Hafsi et al., 2001; Merah et al., 2001b; Monneveux et al., 2005), and under severe stress, to leaf Δ (Hafsi et al., 2001). The objectives of the present study were to investigate the variation in the association between yield, senescence and carbon isotope discrimination under the strong stress conditions of the High-Plateaux of Eastern Algeria.

Material and Methods

Plant material and growth conditions

The study was conducted at experimental fields of the Institut Technique Moyen Agricole (ITMA) of Sétif (5^o 21' W, 36^o 9' S, 1123 m above sea level), Eastern Algeria, during two successive cropping seasons (2001-2002 and 2002-2003). Ten durum wheat cultivars (Table 1) were grown in randomized block design with two replicates. Plots were 10 m x 4 rows with 18 cm row spacing and interplant space of 3 cm. Sowing density was adjusted to 300 g m^{-2}. Sowing was done on December 5 in season 1 (2001-2002) and November 24 in season 2 (2002-2003) while harvesting in both seasons was carried out on June 25. The soil at the experimental site is a rendzin, mollisol (Calcixeroll USDA) up to 0.6 m in depth, containing low organic matter. P (superphosphate 100 kg ha^{-1}) and K (100 kg ha^{-1} were applied to all plots before sowing, while N (urea 150 kg ha^{-1}) was applied at tillage to all plots. Weeds were removed manually as and when required.

Table 1. Brief description of the ten genotypes used in the study

Cultivar	Name	Information
1	Mexicali	CIMMYT cultivar, released in 1975
2	Sooty9/Rascon57	CIMMYT advanced line
3	Nacori	CIMMYT cultivar, released in 1997
4	Waha	CIMMYT/ICARDA line (Sham 1) released in 1986
5	Tilo1/Lotus4	CIMMYT advanced line
6	Yavaros	CIMMYT cultivar, released in 1979
7	Altar	CIMMYT cultivar, released in 1984
8	Dukem12/Rascon21	CIMMYT advanced line
9	Kucuk	CIMMYT cultivar, released in 1984
10	Cado/Boomer33	CIMMYT advanced line

Measurements

The number of days to sowing to heading (DH) was recorded. At maturity 20 spikes were randomly collected and threshed manually to obtain the number of grain per spike (NGS). Grain yield (GY) was determined from a 2.88 m^2 central area. Thousand kernel weight (TKW) was determined from sub-samples taken from harvested grains of each plot.

Leaf senescence was evaluated by numerical image analysis (NIA) according to Hafsi et al. (2000). Four leaves per cultivar were sampled at 300 ^oC day after anthesis and immediately photographed on a black surface between 11:00 and 12:00 solar time with a color digital camera (Sony SSC-C108P, Kyoto, Japan). Images were stored in a JPEG (Joint Photographic Expert Group) prior to downloading onto a PC computer and analyzed using IPP (Image Pro Plus, Version 4, Media Cybernetics, Silver Spring, MA, USA) software. Senescence was expressed as the ratio of senesced area to total leaf area (in per cent).

At anthesis, twenty flag leaves were randomly detached from each plot and oven dried at 80 °C for 48 h. After harvest 10 g of grain were collected from each plot. Leaf and grain samples were ground to a fine powder and composite samples from two replicates was used for carbon isotope composition. The C isotopic ratio ($R={}^{13}C/{}^{12}C$) of samples (R_{sample}) and standard ($R_{standard}$) was determined using an isotope ratio mass spectrometer in the Seibersdorf laboratory of the International Atomic Energy Agency (IAEA), Vienna, Austria. R values were converted to δ (in ‰) using the relation: $\delta^{13}C$ (‰) = $[R_{sample}/R_{standard}-1] \times 1000$. Carbon isotope discrimination (Δ) values were calculated as Δ (‰) = $(\delta^{13}C_a - \delta^{13}C_p) / (1 - \delta^{13}C_p/1000)$ (Farquhar et al., 1989), where $_a$ and $_p$ represent air and plant, respectively.

Data were analyzed using SAS, version 8.1. (SAS Institute 1987, Cary, NC, USA). GLM procedure was used for variance and correlation analysis.

Results

Rainfall was higher in season 1 (217 mm) than in season 2 (162 mm). In season 1, most of the precipitation (80%) occurred during early growth period (December and January), well before anthesis (Fig. 1). Conversely, in season 2, rainfall was distributed evenly during months of January, February and March, near or shortly before anthesis. Season 1 was also characterized by high temperatures during the post-anthesis period and by low minimal spring temperatures, with negative (freezing) temperature occurring during the heading period. GY was affected significantly by season and season x genotype interactions (Table 2). Genotype effect on GY was significant in season 2 but not in season 1.

Mean GY in season 2 was significantly higher than in season 1 (Table 3). In season 1, NGS was particularly low and significantly correlated to GY.

Mean leaf senescence was 73% higher in season 1 than in season 2. The two old cultivars Mexicali and Yavaros showed strong decreases, while the other eight cultivars showed increases in leaf senesence in season 2, compared to season 1. In season 1 average senescence of the five top yielding genotypes (Kucuk, Altar, Sooty9/Rascon57, Yavaros and Tilo1/Lotus4) was less than 45%, while in season 2 the five top yielding cultivars (Yavaros, Waha, Tilo1/Lotus4, Dukem12/Rascon21 and Mexicali) had more than 65% leaf senescence. Senescence was significantly and negatively correlated with grain yield in season 1, but not in season 2 (Fig. 2).

Highly significant effects of genotype, season and genotype x season were found on leaf and grain carbon isotope discrimination. ΔL was 20.0 and 14.3% higher than ΔG in season 1 and 2, respectively (Table 5). Mean ΔL and ΔG were significantly higher in season 1 than in season 2. There was no relationship between ΔG and grain yield. However, a significant positive correlation was noted in season 2 by eliminating the cultivars Nacori and Dukem12/Rascon21 that had the lowest yields (Fig. 3).

Discussion

Effects of climatic conditions on yield, senescence and carbon isotope discrimination

Lower GY and higher senescence rates in season 1 compared to season 2 may be attributed to climatic conditions (rainfall and temperature). Sharp increase in temperature during grain filling stage in season 1 is likely to have accelerated senescence. Lower grain yield is probably the consequence of a lower grain setting caused by freezing at heading and drought and high temperatures around anthesis. The low number of grains per spike and its strong correlation with grain yield in season 1 supports this hypothesis. ΔL was significantly higher in season 1 than in season 2. Leaf Δ is mainly controlled by stomatal opening (Farquhar and Richards, 1984) and consequently largely determined by pre-anthesis conditions that allow to maintain high stomatal conductance (Morgan et al., 1993). More water supply due to higher rainfall in early growing season 1 may have led to higher stomatal conductance and consequently higher leaf Δ.

Table 2. Variance analysis, mean and standard-deviation (SD) for grain yield (GY), leaf carbon isotope discrimination in flag leaf at anthesis (ΔL_a) and in grain at maturity (ΔG_m)

	GY	ΔL_a	ΔG_m
σ² genotype	2.41NS	4.53***	2.20***
σ² season	274.13***	666.82***	97.92***
σ² genotype x season	29.58***	70.76***	11.77***
Season 1			
Mean	0.65^b	17.28^a	14.30^a
SD	0.14	0.40	0.13
σ² genotype	2.49NS	2.54NS	21.50***
Season 2			
Mean	1.43^a	15.11^b	13.35^b
SD	0.95	0.083	0.044
σ² genotype	4.89**	19.95***	43.65***

*** significant at $P = 0.001$; NS, non significant; mean values on the same column without a common letter are significantly different ($P < 0.05$) according to the Duncan comparison test.

Table 3. Average value and correlation with yield for number of grains per spike and thousand kernel weight in season 1 and season 2

	Number of grains per spike	Thousand kernel weight (g)
Season 1		
Mean	10.62[a]	36.00[a]
Correlation with grain yield	0.860***	0.373NS
Season 2		
Mean	16.52[b]	34.39[a]
Correlation with grain yield	0.600NS	0.624*

NS, non significant; Mean values on the same column without a common letter are significantly different ($P < 0.05$) according to the Duncan comparison test.

Table 4. Variation of carbon isotope discrimination in flag leaf at anthesis (ΔL_a) and in grain at maturity (ΔG_m) and correlation with grain yield in seasons 1 and 2.

	ΔL_a	ΔG_m
Season 1		
Mean	17.16[a]	14.30[a]
Correlation with grain yield	-0.065NS	0.147NS
Season 2		
Mean	15.21[b]	13.31[b]
Correlation with grain yield	-0.518NS	0.061NS

NS, non significant; Mean values on the same column without a common letter are significantly different ($P < 0.05$) according to the Duncan comparison test.

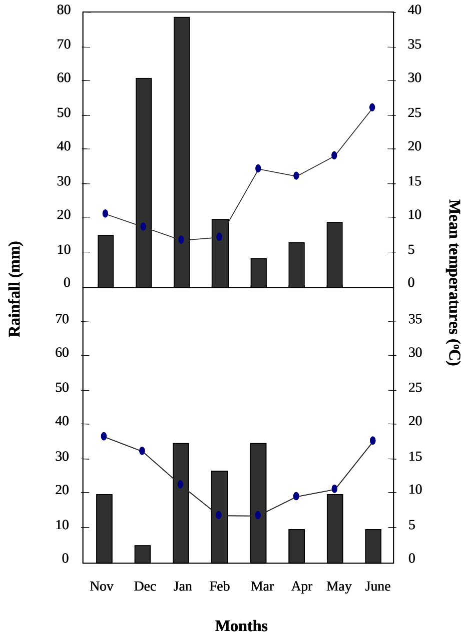

Fig. 1. Climatic conditions during seasons

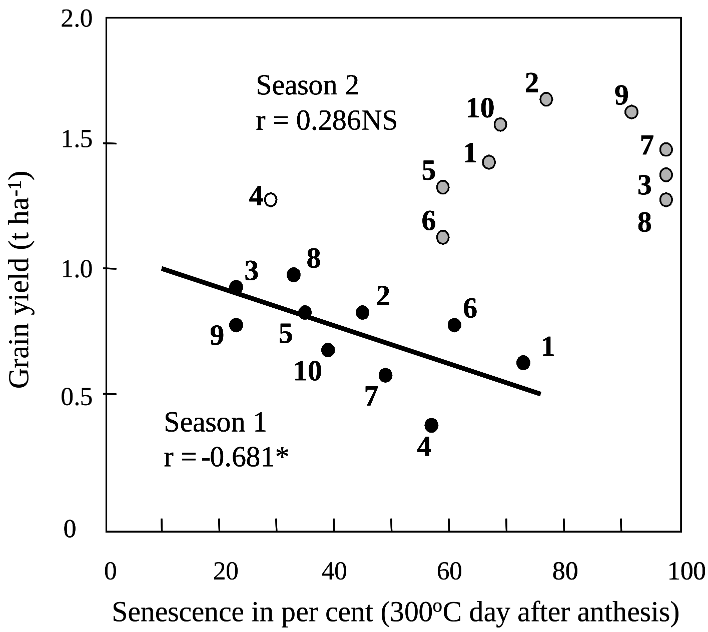

Fig. 2. Relationship between senescence the two cropping and grain yield (seasons1 and 2)

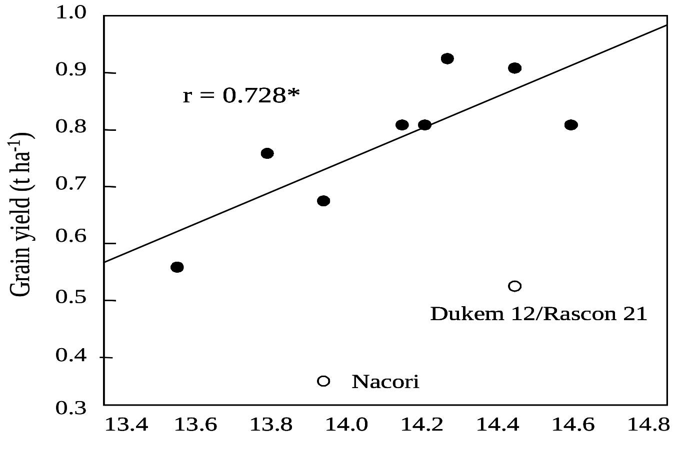

Fig. 3. Relationship between grain carbon isotope discrimination and grain yield in season 2

The higher difference in Δ between leaf and grain in season 1 (2.4 ‰) compared to season 1 (1.9 ‰) is in good accordance with the strong stress experienced by the crop after anthesis. The significantly higher grain Δ in season 1 is probably related to a higher contribution to grain filling of C products having high Δ values. Grain Δ is influenced both by stomatal conductance and re-mobilization from vegetative parts of the plant (Hannachi et al., 1996; Hafsi et al., 2001; Merah et al., 2001b). The contribution of re-mobilization dramatically increases with drought (Loss and Siddique, 1984). Products filling the grain are likely to be originated from vegetative organs and, having been synthesized under optimal conditions, have a higher Δ. Slafer and Araus (1998) and Royo et al. (1999) also suggested that Δ under severe terminal drought is defined early in the crop cycle, photoassimilates produced before anthesis playing a major role in determining grain yield.

Relationship between grain yield, senescence and carbon isotope discrimination

The lack of correlation between Δ values and grain yield is in agreement with earlier findings from Hafsi et al. (2003) and Araus et al. (2003) and is likely to be due to a strong contribution of pre-anthesis assimilates to yield together with a sink limitation of yield, breaking the association observed between Δ and yield under post-anthesis water stress by several authors (Araus et al., 1998; Merah et al., 2001b; Monneveux et al., 2005). Heading stage coincided with strong drought and frost (particularly in the first season) that strongly reduced potential grain number. Significant correlation between grain yield and GPE and significant correlation observed in season 2 between grain yield and Δ after excluding the genotypes (Dukem and Nacori) in which ear fertility was more affected, support this hypothesis.

Senescence showed a significant negative correlation with grain yield in season 1, in good agreement with Rawson et al. (1983) and Ellen (1987). Contrary to these findings many studies have demonstrated that delayed senescence delays remobilization and leads to reduced grain weight (Yang et al., 1997; Zhu et al., 1997). Yang et al. (2001) confirmed that association between grain yield and senescence highly depends on climatic conditions. There was no clear relationship between senescence and carbon isotope discrimination, suggesting that senescence is poorly related with transpiration efficiency.

The results of the present study confirmed that the association between senescence and yield in wheat highly depends on environmental conditions. They also showed that the relationship between carbon isotope discrimination and grain yield reported by many authors under Mediterranean climate is not confirmed under severe stress conditions, particularly when sink capacity is affected.

Acknowledgements. The authors would like to thank Dr. W. Pfeiffer (CIMMYT) for providing the seeds used in the experiments. Carbon isotope discrimination analysis was funded by IAEA, through its technical contract No. 11873/RBF.

References

Adamsen F.J., Pinter P.J., Barnes E.M., Lamorte R.L., Wall G., Leavitt W., Kimball B.A. 1999. Measuring wheat senescence with a digital camera. *Crop Sci*. 39: 719-724.

Araus J.L, Amaro T., Casadesus J., Asbati A., Nachit M.M. 1998. Relationship between ashcontent, carbon isotope discrimination and yield in durum wheat. *Aust. J. Plant Physiol*. 25: 835-842.

Araus J.L., Villegas D., Aparicio N., Garcia del Moral L.F., El Hani S., Rharrabti Y., Ferrio J.P., Royo C. 2003. Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. *Crop Sci*. 43: 170-180.

Clarke T.R. 1997. An empirical approach for detecting crop water stress using multispectral airborne sensors. *Horttechnology* 7: 9-16.

Dymond J.R., Trotter C.M. 1997. Directional reflectance of vegetation measured by a calibrated digital camera. Optics 18: 4314-4319.

Ellen J. 1987. Effects of plant density and nitrogen fertilization in winter wheat (*Triticum aestivum* L.): I. Production pattern and grain yield. Neth. *J. Agric. Sci*. 35: 137-153.

Farquhar G.D., Richards R.A. 1984. Isotopic composition correlates with water use-efficiency of wheat genotypes. *Aust. J. Plant Physiol* 11: 539-552.

Farquhar G.D., Ehleringer J.R., Hubick K.T. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. *Plant Physiol. Plant Mol. Biol*. 40: 503-537.

Gebbing T., Schnyder H. 1999. Pre-anthesis reserve utilization for protein and carbohydrates synthesis in grains wheat. *Plant Physiol*. 121: 871-878.

Guo T.C., Wang C.Y., Zhu Y.J., Wang H.C., Li J.X., Zhou J.Z. 1998. Effects of high temperature on the senescence of roots and tops of wheat plants in the later stage. *Acta Agron. Sinica* 24: 957-962. [in Chinese, English summary]

Hafsi M., Mechmeche W., Bouamama L., Djekoune A., Zaharieva M., Monneveux P. 2000. Flag leaf senescence, as evaluated by numerical image analysis, and its relationship with yield under drought in durum wheat. *J Agronomy and Crop Sci*. 185: 275-280.

Hafsi M., Monneveux P., Merah O., Djekoune A. 2001. Discrimination isotopique du carbone et rendement du blé dur dans les hautes-plaines sétifiennes, Algérie. *Sécheresse* 12: 37-43. [in French, English summary].

Hafsi M., Pfeiffer W.H., Monneveux P. 2003. Flag leaf senescence, carbon isotope discrimination in durum wheat under semi-arid conditions. *Cereal Res.*

Comm. 31: 161-168.

Hannachi I., Deleens E., Gate P. 1996. Nitrogen and carbon isotope composition of wheat grain: alteration due to sink source modifications at flowering. Rapid Comm. Mass Spectrum. 19: 979-986.

Loss S.P., Siddique K.H.M. 1984. Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. *Adv. Agron.* 52: 229-276.

Merah O., Deléens E., Monneveux P. 2001a. Relationships between flag leaf carbon isotope discrimination and several morphophysiological traits in durum wheat under Mediterranean conditions. *Environ. Exp. Bot*. 45: 63-71.

Merah O., Deléens E., Teulat B., Monneveux P. 2001b. Productivity and carbon isotope discrimination in durum wheat organs under a Mediterranean climate. C R Acad. Sci. Paris 324: 51-57.

Mi G.H.,Tang L., Zhang F.S. 1999. Nitrogen uptake and translocation during grain formation of two wheat cultivars with contrasting maturity appearance. *J. China Agric. Univ.* 4: 53-57. [in Chinese, English summary]

Monneveux P., Reynolds M. P., Trethowan R., González-Santoyo H., Peña R.J., Zapata F. 2005. Relationship between grain yield and carbon isotope discrimination in bread wheat under four water regimes. *Eur. J. Agron*. 22: 231-242.

Morgan J.A., LeCain D.R., McCaig T.N., Quick J.S. 1993. Gas exchange, carbon isotope discrimination and productivity in winter wheat. *Crop Sci*. 33: 178-186.

Pajević S., Krstić B., Stanković Z., Plešnicar M., Dencić S. 1999 Photosynthesis of flag leaf and second wheat leaves during senescence. *Cereal Res. Comm*. 27: 155-162.

Paulsen G.M. 1994. High temperature response of crop plants. In: Physiology and determination of crop yield. Boote K.J. et al. eds. pp 365-389. ASA, CSSA and SSSA, Madison, WI.

Rawson H.M., Hindmarsh J.H., Fisher R.A., Stockman Y.M. 1983. Changes in leaf photosynthesis with plant ontogeny and relationships with yield per ear in wheat cultivars and 120 progeny. *Aust. J. Plant Physiol.* 10: 503-514.

Royo C., Voltas J., Romogosa I. 1999. Remobilization of pre-anthesis assimilates to the grain and for grain only and dual-purpose (forage and grain) triticale. *Agron. J*. 91: 312-316.

SAS Institute, 1987. SAS/STAT user's guide, version 6. SAS Inst., Inc., Cary, NC.

Slafer G.A., Araus J.L. 1998. Keynote address: Improving wheat responses to a biotic stress . InProceeding of the 9th Int. wheat genetics symposium. Sinkard A.E. ed. Vol 1, Saskatoon. Univ. of Saskatchwan, Extension press. Saskatoon , Canada.

Yang J., Wang Z., Zhu Q. 1997. Photosynthetic characteristics, dry matter accumulation and its translocation in inter-specific hybrid rice. *Acta Agron. Sincia.* 23: 82-88. [in Chinese, English summary].

Yang J., Wang Z., Zhu Q., Liu L. 2001. Water deficit induced senescence and its relationship to remobilization of pre stored carbon in wheat during grain filling. *Agron. J*. 93: 196-206.

Zhu Q., Zhang Z., Yang J., Wang Z. 1997. Source-sink characteristics related with the yield of inter-subspecific hybrid rice. *Sci. Agric. Sinica* 30: 52-59. [in Chinese, English summary].