International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 8(1): 135-143 March 2015
DOI Number: 10.5958/2230-732X.2015.00018.2

GENETICS AND PLANT BREEDING

Study of genetic variation among soybean [*Glycine Max* (L.) Merrill] accessions in CID values

Mithlesh Kumar*[1] and Sanjay Kumar Lal[1]

[1]Division of Genetics, Indian Agricultural Research Institute, New Delhi, 110 012, India.
Present addres: [1]Department of Genetics and Plant Breeding, C.P. College of Agriculture, S.D. Agricultural University, Sardarkrushinagar, 385 506, India.

*Corresponding author: mithleshgenetix@gmail.com

Paper No. 302 Received: 03 February 2015 Accepted: 3 March 2015 Published: 25 March 2015

ABSTRACT

A set of 91 diverse soybean accessions were analysed for 10 morphological traits with special reference to water use efficiency using carbon isotope discrimination (CID) technique. The coefficient of variation for CID values was found to be 3.36%. There is negative correlation between $\Delta^{13}C$ values (CID values) and WUE. The genotypes which registered lower values of $\Delta^{13}C$ are more water efficient than the genotypes with higher $\Delta^{13}C$ values. The range of $\Delta^{13}C$ values observed in this experiment are slightly lower than that reported in sugar beet ($\Delta^{13}C$ 17.66‰ to 22.96‰), (Rajabi *et al.*, 2009). Genotype UPSL309 has highest CID value (22.91‰) indicating low WUE and genotype DS9813 has lowest CID value (19.95 ‰) with high WUE. In present study randomly classified genotypes as high WUE (CID less than 20.5 ‰), medium WUE (CID between 20.6 to 22.4 ‰) and low WUE (CID above 22.5 ‰). There were 7 genotypes which were found to show high WUE, and 13 exhibited low WUE whereas, rests of the 71 genotypes were medium in WUE.

Highlights

- In 91 diverse soybean accessions there are 7 which show high WUE, 13 exhibited low WUE and rest 71 accessions were medium WUE.

Keywords : *Glycine max* (L) Merrill, Water Use Efficiency (WUE), CID, genotypes

More than 90% of the soybean is grown as a rainfed crop in India (Holt *et al.*, 1997). Therefore, the crop suffers from frequent incidence of drought, which in severe case leads to death of plants resulting in immense yield losses. Soybean season spreads over four months. Usually, it starts with the onset of monsoon in the second fortnight of June and ends in the month of September. However, it might extend into the month of October, if there is a delay in sowing. The analysis of rainfall and evapotranspiration data shows that there is recurrent drought during the month of September, which affects grain formation resulting in low yield (Lal *et al.*, 2009). There is ample rainfall during the months of July and August; therefore, the crop has to survive on residual moisture during the month of September. Under such conditions breeding for increased water use efficiency (WUE) has been shown to be the most potent strategy. The WUE of a plant is generally defined as the amount of biomass accumulated per unit of water used (Manavalan *et al.*, 2009). WUE has been adopted to assess drought tolerance in various soybean cultivars as a direct yield scoring method (Liu 2009). The positive association between WUE and total biomass yield in drought environment suggests that improvement of the WUE of a crop plant result in superior yield performance if a high harvest index (HI) can be maintained (Wright 1996). Water consumption and its efficient use by crops are related to yield. In soybean, a widely accepted equation for grain yield (Y) under water limited conditions is a function of three components, namely the amount of water transpired (T), water use efficiency (WUE) and harvest index (HI); Y = T × WUE × HI (Turner *et al.,* 2001). Measuring WUE is a highly tedious and almost impossible particularly, in large breeding population. Development of carbon isotope discrimination (CID) technique by Farquhar *et al.*, (1982) as an alternative for measuring WUE in C_3 species has made it possible to measure WUE in a large breeding population. Usefulness of CID for measuring WUE in soybean has already been reported (Mian *et al.,* 1996; Specht *et al.,* 2001). Ash content has been proposed as cheap and easily determined surrogates of Delta (Δ) (Tsialtas *et al.,* 2002).This work needs to be extended in Indian context. Therefore, identification and incorporation of WUE in high yielding background is needed to reduce the losses due to drought. The present attempt in soybean to screen a set of soybean germplasm accessions for WUE using CID technique.

Materials and Methods

Study area

The experiment was conducted at experimental farm of Division of Genetics, Indian Agricultural Research Institute, Pusa Campus, New Delhi.

Experimental materials

A set of 91 accessions comprising of released varieties, advanced breeding lines, exotic collections and indigenous collections were grown in an augmented design (Federer 1956) using 6 checks (PS 416, PS 1024, DS 9814, SL 444, EC 472183 and DS 9712) in a 5 m single row plot. Standard agronomic practices recommended for the soybean crop was followed.

Estimation of water use efficiency using CID values

For assessing the water use efficiency, carbon isotope discrimination (CID) method suggested by Farquhar *et al.,* (1982) was followed. Leaf samples of sixty days old plants under well watered conditions were collected from five randomly tagged plants in each accession. The leaf samples from five plants were bulked together, thus samples were divided into two replications for each accession.

Determination of delta ^{13}C in plant samples using Flash Elemental Analyzer (Flash-EA) and Isotope-Ratio Mass Spectrometry (IRMS)

Sample preparation

The leaf samples were collected sixty days after sowing under well watered condition. The leaf samples were oven dried at 80°C for 3 days. The samples were completely dried when the biomass reached a constant weight on subsequent weighing. Dried samples were then powdered in a mortar and pestle. Care was taken to avoid any contamination from other samples by washing the mortar and pestle with alcohol after grinding each sample.

Table 1. Morphological variation for different quantitative traits among the 91 diverse accessions of soybean

	SLA (mg/cm^2)	Leaf ash (mg/g leaf dry wt.)	CID (‰)	Days to 50 % flower	Days to maturity	Plant height (cm)	Branches/ plant	Pods / plant	Seeds/ pod	Seed yield/ plant
N	91	91	91	91	91	91	91	91	91	91
Maximum	0.008306	0.139	22.91857	55	120	122.2	8.6	108.36	3	23.8
Minimum	0.001334	0.073	19.95025	37	101	28.8	1.2	25.6	1.6	1.6
Mean	0.004774	0.096	21.69132	45.33	107.29	68.48	3.99	61.98	2.15	8.14
CV (%)	24.03	11.53	3.36	9.43	3.99	25.23	29.47	33.23	11.18	50.42

Legend: Where, ‰ indicate unit of CID values

Table 2. Top ten Soybean accessions with low CID values

Accession No.	CID(‰)	Leaf ash (mg/g leaf dry wt.)	Days to 50% to flower	Days to maturity	Plant height (cm)	Branches/ plant	Pods / plant	Seeds/ pod	Seed yield/ plant
EC471999	19.95025	0.093	43	112	50.2	3.8	66	2.4	8.6
EC472107	20.01834	0.106	51	103	85.6	5	70.6	2.2	6.2
UPSL340-A	20.0634	0.095	52	120	93.8	5	74	2.2	9.4
EC472137	20.23956	0.098	52	110	80.6	4.6	90.6	1.8	5
DS9812	20.33614	0.095	46	108	62	2.8	42.4	2.4	4
EC472143	20.39487	0.097	51	109	90.2	3.8	102.8	2.2	4.8
EC472227	20.48268	0.117	52	112	71.8	4	58.8	2.2	5.2
EC547195	20.57661	0.098	44	105	63.6	4	78.8	2.6	11.8
UPSV24	20.60277	0.093	52	116	122.2	4.2	82.4	2.2	8.2
DS9813	20.73321	0.081	43	106	64	5	84.6	2.2	19.6

Legend: Where, ‰ indicate unit of CID values

Flash combustion and ^{13}C analysis

According to methodology given by Brand (1996) samples were combusted under controlled conditions in the presence of appropriate catalysts to generate CO_2 gas that can be analyzed by IRMS for m/z ratios. To achieve this, very small quantity, typically in the rage of 0.6 to 0.8 mg of the samples in affine powder are weighed accurately into silver boats and combusted in the Flash elemental analyzer (NA 1112, Carlo Erba, Italy) interfaced to an Isotope Ratio Mass Spectrometer (IRMS; Delta-Plus, Thermo-Finnigan, Bremen, Germany) via a continuous flow device (Conflo-III). The crimped boats with the samples are placed sequentially in the sample carousel of the auto sampler. The auto sampler drops the samples onto the oxidation reactor whose timing and speed is determined by a software control. The samples are once fall into the oxidation reactor maintained at a high temperature of 102^0C is immediately combusted which is accelerated with a pulse of pure oxygen to aid the combustion process. Transiently, the temperature of the reactor at the point of flash combustion is increased up to 188^0C. At this high temperature, the organic sample is completely oxidised to produce CO_2, traces of CO, N_2O and dH_2O. The oxidation process is catalyzed by the two metal oxides in the reactor. The chromium oxide and Silver coated cobaltous-cobaltic oxide oxidise traces of CO to CO_2 and hence ensure quantitative conversion of the biomass to its gaseous constituents. These gases are then swept into the reduction reactor by helium carrier gas. The reduction reactor contains reduced copper in quartz tubes heated to 68^0C. Here the N_2O is reduced to N_2 gas and excess oxygen not used for combustion is absorbed. The resultant gases (CO_2, N_2 and H_2O) are then flushed through magnesium per chlorate trap to remove water. The pure CO_2 and N_2 gases after passing through a gas chromatography (GC) column (5A molecular sieve) and a thermal conductivity detector (TCD) are introduced into the ion source of the IRMS by the conflo interface (Nataraja *et al.,* 2009).The IRMS determines the m/z ratio of the CO_2 hence generates the molar ratio of the heavy to lighter isotope ($^{13}C/^{12}C$). This ratio is compared with that of the international isotopic standard *i.e.* PDB standard to compute the delta ^{13}C as follows.

Table 3 Top ten Soybean accessions with high CID values

Accession No.	CID (‰)	Leaf ash (mg/g leaf dry wt.)	Days to 50% to flower	Days to maturity	Plant height (cm)	Branches per plant	Pods per plant	Seeds per pod	Seed yield per plant
EC457323	22.91857	0.090	37	106	58.60	5	95.4	2.4	13.8
SL525	22.86952	0.086	44	105	86.80	3.2	46.8	2.2	8.2
UPSL244	22.81997	0.098	51	110	99.80	2.8	53.4	3	4.6
PS1374	22.79143	0.106	45	102	56.00	5	76.6	1.8	5.4
EC471805	22.78940	0.094	47	104	39.60	3.6	37.8	2.2	7.4
UPSL309	22.76438	0.095	50	117	82.00	4.2	30.4	2	2.8
SL 67	22.69817	0.090	43	109	58.60	3.6	68.6	2	8.6
PK 292	22.68194	0.117	38	106	65.20	3.4	61.8	2.2	9.2
V9	22.68054	0.099	46	106	106.80	8.6	103.8	2.4	17.8
EC483062	22.53638	0.102	41	110	56.20	3.6	108	2	12.4

Legend: Where, ‰ indicate unit of CID values

$\delta\ ^{13}C_{sample} = (R_{sample}/\ (R_{PDB}-1))$ x 1000. It is well known that the isotopic composition of plant biomass is a function of the isotopic fraction that occurs at different stages during the photosynthetic carbon fixation process. These fractionations broadly occur at the stomatal diffusive stage and then at the site of carboxylation by RuBisCO enzyme.Combining these two steps of fractionation, the carbon isotope discrimination ($\Delta\ ^{13}C$) can be computed as follows: $\Delta^{13}C$ =a + (b-a) p_i/p_a Where, 'a' and 'b' are fractionations that occur at stomatal diffusive step and carboxylation by RuBisCO, respectively(Condon et al.2004). The p_i and p_a are the partial pressure of CO_2 in the intercellular spaces and the ambient air, respectively. The fractionation that occur during diffusion and biochemical reaction are considered as constants (-4.4‰ and -29‰, respectively). Therefore, the carbon isotope discrimination is function on the ration of the CO_2 partial pressure (p_i/p_a). Alternatively, the $\Delta^{13}C$ can also be computed from the following equation given by Farquhar *et al.*, (1989); $\Delta^{13}C$ (‰) = ($\delta_a{}^{13}C$- $\delta_p{}^{13}C$) / (1+ $\delta_p{}^{13}C$/1000) Where, $\delta_a{}^{13}C$ is the isotopic composition of the ambient air corrected against the PDB Standard. In an unpolluted air, the $\delta_a{}^{13}C$ is considered as -8‰. $\delta_p{}^{13}C$ is the isotopic composition of the organic simple corrected against the PDB Standard as determined by the IRMS. All stable isotope measurements were made at the National Facility for Stable Isotope Studies, Department of Crop Physiology, UAS, Bangalore. Data were also collected on specific leaf area (SLA), leaf ash, days to 50% flowering, days to maturity, pods per plant, branches per plant, seeds per pod, seed yield per plant and plant height for five randomly tagged plants in each accession. Specific leaf area was taken as the ratio of leaf dry weight of leaf piece of one square cm area (Garnier et al.1997). For leaf ash content leaves were dried in the oven at 70^0C temperature for 72 hours. The dry leaves were grinded into fine powder using pestle and mortar. One gram of dried leaf powder was put into the crucible and kept into the muffle furnace at 550^0C temperature for 2 hours. Variability in the ten quantitative traits was observed in terms of mean, minimum, maximum, range and coefficient of variation (CV).

Table 4. Soybean accessions showing low, medium and high WUE

High CID (Low WUE) value accessions	Medium CID (Medium WUE) value accessions	Low CID (High WUE) value accessions
EC389179, EC457323, EC483062, V9, PK292, PS1374, SL444, SL525, SL528, SL637, UPSL244, EC457331, UPSL309	DS9801, DS9813, DS9816, DS9819, DS9821, PS1042, DS9822, DSb6, EC390981, EC456646, EC547191, EC457321, EC472183, EC457381, EC457397, EC457403, EC457470, EC471867, EC471994, EC472024, EC472129, PS1024, EC472130, G2601, HIS01, IC141446, JS(SH)98-22, L291, L440, M1094, NRC45, NRC47, PS1024, SL444, PK1060, SL444, PK1080, PK1135, PK1180, PK1197, PK1241, PK1243, PS416, PK1251, PK1259, PK1284, PK1347, V8, DS9804, DS9814, PS1392, PS1394, SL295, SL459, PS1042, SL633, UPSL303, UPSL332, UPSV24, DS960, DS9719, DS9802, PS1024, EC44303, EC457155, EC457183, EC457196, EC457266, EC457285, EC457312, SL444	EC471999, EC472107, EC472137, EC472143, EC472227, UPSL340-A, DS9812

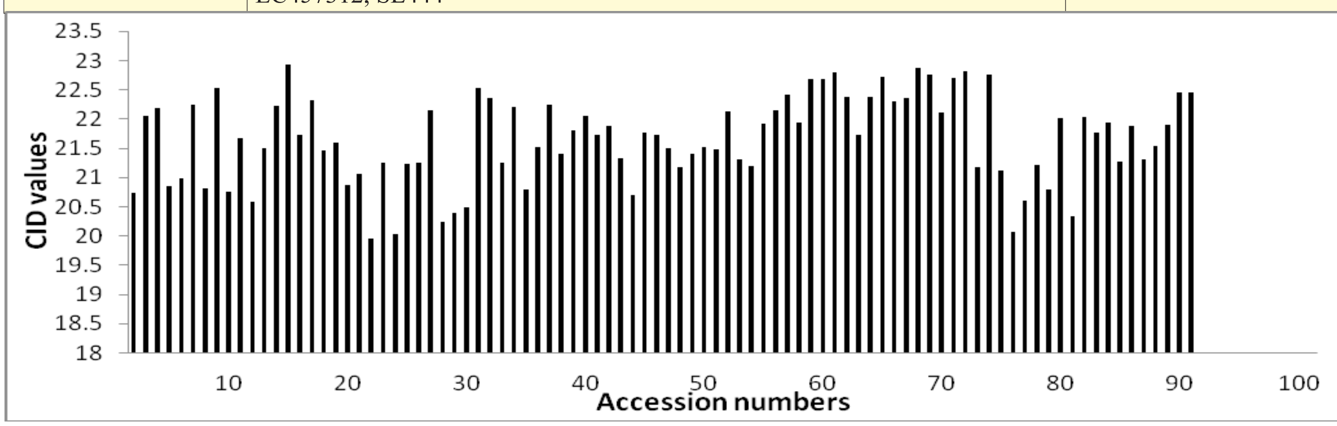

Figure 1. Variability for CID in 91 accessionss of soybean

lowest and Highest CID value efficient genotypes

Figure 2. Genotype DS 9813 (High WUE) Genotype UPSL 309 (Low WUE)

Results and Discussion

Variability in morphological traits

Variability in the ten quantitative traits were observed in terms of mean, minimum, maximum, range and coefficient of variation (CV %) and are summarized in Table 1. The coefficient of variation was found to be the lowest for water use efficiency (3.36%) and highest for seed yield (50.42%). The variability was also very poor in maturity (3.99%), days to flower (9.43%), seeds per pod (11.18%) and leaf ash (11.53%). Contrary to this the variability was reasonably high in the traits namely specific leaf area (24.03%), plant height (25.23%), branches per plant (29.47%) and pods per plant (33.23%).

Variability for water use efficiency

The CID values of accessions ranged from 19.95 ‰ to 22.91 ‰ (fig. 1) and coefficient of variation of CID values was 3.36%. In a study involving 24 soybean genotypes, WUE found to range from 2.7 g dry matter/kg water to 3.4 g dry matter/kg water used (Hufstetler *et al.,* 2007). As negative correlation has been registered between CID values and water use efficiency (Hubick *et al.*, 1988), the genotypes which registered lower CID values (Table 2) are more water efficient than genotypes which have higher CID values(Table 3). WUE is influenced by the photosynthetic capacity and stomatal conductance. It has been proposed that the aperture of stomata could be regulated in such a way that a partial closure of stomata at a certain level of soil water deficit might lead to an increase in WUE (Liu *et al.,* 2005). Though the observed CV for CID values was low (3.36%) as compared to the values reported in other crops such as sugar beet- CID 17.66‰ to 22.96 (Rajabi *et al.,* 2009). Genotype UPSL309 has highest CID value (22.91‰) indicating low WUE and genotype DS9813 has lowest CID value (19.95 ‰) showing high WUE (fig. 2). In present study we randomly classified genotypes as high WUE (CID less than 20.5 ‰), medium WUE (CID between 20.6 to 22.4 ‰) and low WUE (CID above 22.5 ‰) in table-4. There were 7 genotypes which were found to show high WUE, and 13 exhibited low WUE whereas rests of 71 genotypes were medium in WUE.A close look at the genotypes showing low CID values reveals that only two genotypes namely DS9813 and EC547195 out of ten have high seed yield per plant (Table 2). Genotype DS9813 registered the highest yield (19.6g) with all desirable characters such as early maturity (106 days), medium plant height (64 cm), high pods per plant (84.6) and high seeds per plant (2.2).Following DS9813 another genotype EC547195 which showed low CID value and high yield per plant (11.8g) also exhibited all desirable traits such as early maturity (105 days), moderate plant height (63.6 cm), high pods per plant (78.8) and high seeds per pod (2.6). Rest of the eight genotypes showing low CID value were poor yields may be because of conservative growth habit. As CID is a very expensive technique, workers have tried to use specific leaf area (SLA) as a cheap alternative for CID. Specific leaf area which is indirect measure of leaf expansion. Higher SLA represents larger surface area for transpiration and hence, SLA and WUE would be inversely related. In the present study, the variation for specific leaf area was found to be very high (CV 24.03%). Leaf ash content has also been tried as an alternative to CID value. Apart from maturity correlation with plant height, specific leaf area and leaf ash has also been reported in soybean (Mian *et al.,* 1996). Mian *et al.,* (1996) reported negative correlation (r = -0.40) between water use efficiency (WUE) and leaf ash in soybean. Therefore a correlation between CID and leaf ash content has also been used a cheap alternative to CID. We made an attempt to see if there was any correlation between CID values and leaf ash content. In the present study, variability for leaf ash content was low (CV 11.53%). Low CID genotypes are generally associated with the conservative crop growth rate, if the differences in CID in the absence of soil deficit are results of differences in stomatal conductance (Condon *et al.,* 2002). Genotypes with lower stomatal conductance will tend to have higher WUE and lower CID if the WUE is associated with lower photosynthetic rate per unit area and consequently a slower rate of crop growth (Condon *et al.,* 2004).

In the absence of the knowledge of photosynthetic rate and stomatal conductance the usefulness of low CID genotypes is doubtful. Therefore the future studies should be taken up with the limited number of genotypes with high, low and medium CID values to study the variation in photosynthetic capacity and stomatal conductance of these selected lines. However, a recent report by Gilbert *et al.,* (2011) in soybean, it has been demonstrated that genotypic differences in stomatal conductance had the greatest effect on CID (26% variation when well watered) and was uncorrelated with the effect of photosynthetic capacity on CID. This suggest that it is possible to employ a selection strategy of breeding water-saving soybeans with high photosynthetic capacities to compensate for otherwise reduced photosynthesis in genotypes with lower stomatal conductance.

Acknowledgements

First author sincerely acknowledges the Indian Council of Agricultural Research (ICAR) for providing the financial assistance in the form of Junior Research Fellowship (JRF) and Post-Graduate School, Indian Agricultural Research Institute (IARI), New Delhi for providing the necessary facilities for his Master's degree programme. The authors also acknowledge the assistance provided by the vice-Chancellor of the S.D. Agricultural University, Sardarkrushinagar (Gujarat) for preparation of this manuscript.

References

Brand WA (1996) High precision isotope ratio monitoring techniques in mass spectrometry. *Journal of Mass Spectrom* **31**: 225-235

Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2002) Improving intrinsic water-use efficiency and crop yield. *Crop Science* **42**: 122-131

Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2004) Breeding for high water-use efficiency. *Journal of Experimental Botany* **55**: 2447-2460

Farquhar GD, O'Leary MH, Berry A (1982) On the relationship between carbon isotope discrimination and intercellular carbon dioxide concentration in leaves. *Australian Jounal of Plant Physiology* **9**: 121-137

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**: 503-537

Federer WT (1956) Augmented (or Hoonuiaku) designs, Hawaiian Planter's Records **55**: 191-208

Garnier E, Cordonnier P, Guillerm JL, Sonie L (1997) Specific leaf area and leaf nitrogen concentration in annual and perennial grass species growing in Mediterranean old-fields. *Oecologia* **111**:490-498

Gilbert ME, Zwieniecki MA, Holbrook NM (2011) Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. *Journal of Experimental Botany* **62**: 2875-2887

Holt DA, Kauffman HE, Bellasco C, Bhatnagar PS, Galerani P, Haun S, Roessing A (1997) In proceedings of the conference on meeting the demand for food in the 21st century; challenges and opportunities for Illinois Agriculture

Hubick KT, Farquhar GD, Condon AG, Richards RA (1988) Carbon isotope fractionation and plant water-use efficiency. In: Rundel PW, Ehleringer JR, Nagy KA (ed) Stable isotopes in ecological research. Springer-Verlag, New York

Hufstetler EV, Boerma HR, Carter TE, Earl HG (2007) Genotypic variation for three physiological traits affecting drought tolerance in soybean. *Crop Science* **47**: 25-35

Lal SK, Sapra RL, Talukdar A (2009) Soybean ideotype for moisture stress areas of central India. *Journal of Oilseeds Research* **26**: 138-140

Liu X (2009) Drought. In: Lam HM, Chang R, Shao G, Liu Z(ed) Research on tolerance to stresses in Chinese soybean. China agricultural press, Beijing.

Liu Y, Gai JY, Lu HN, Wang YJ, Chen SY (2005) Identification of drought tolerant germplasm and inheritance and QTL mapping of related root traits in soybean (Glycine max (L.) Merrill). *Acta Genetica Sinica* **32:** 855-863

Manavalan PL, Guttikonda SK, Tran LSP Nguyen HT (2009) Physiological and molecular approaches to improve drought resistance in soybean. *Plant Cell Physiology* **50**: 1260-1276

Mian MAR, Mailey MA, Ashley DA, Wells R, Carter TE, Parrott WA, Boerma HR (1996) Molecular markers associated with water use efficiency and leaf ash in soybean. *Crop Science* **36**: 1252-1257

Nataraja KN, Madhura JN, Mohan RB, Sheshshayee MS (2009) Laboratory manual training course on "Physiological and molecular basis of plant adaptations to drought". Department of crop physiology, University of Agricultural Sciences, GKVK Campus, Bangalore 560 065

Rajabi A, Ober E, Griffiths H (2009) Genotypic variation for water use efficiency, carbon isotope discrimination, and potential surrogate measures in sugarbeat. *Field Crops Research* **112**: 172-181

Specht JE, Chase K, Macrander M, Graef GL, Chung J, Markwell JP, Germann M, Orf JH Lark KG (2001) Soybean response to water: a QTL analysis of drought tolerance. *Crop Science* **41**: 493-509

Tsialtas JT, Kassioumi M, Veresoglou DS (2002) Evaluating leaf ash content and potassium concentration as surrogates of carbon isotope discrimination in grassland species. *Journal of Agronomy Crop Science* **188**: 168-175

Turner NC, Wright GC, Siddique KHM (2001) Adaptation of grain legumes (pulses) to water-limited environments. *Advances in Agronomy* **71**: 193-223

Wright, G (1996) Review of ACIAR selection for water use efficiency in legumes project recommends further research. ACIAR Food Legume Newsletter. 2-3