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Assessment of genotypic variation in soybean for water use efficiency (WUE) using Carbon Isotope Discrimination (CID) technique

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Abstract

Genotypic variations were analyzed in a set of 91 diverse soybean accessions for 10 morphological traits and water use efficiency (WUE) using Carbon Isotope Discrimination (CID) technique. The Euclidean distance was used as a measure of dissimilarity among the accessions and Ward's method was applied to classify the genotypes and draw the dendrogram. Moderate level of variability for CID (19.95 to 22.91%) was observed among the genotypes. A significant negative correlation (–0.241) between water use efficiency (WUE) and days to maturity was observed. The genotypes in two clusters (Cluster I and V) differed significantly for most of the traits including CID (20.76 Vs 22.43%), days to 50% flowering (50.75 Vs 39.43), days to maturity (117.25 Vs 107.67), plant height (94.70 Vs 63.50 cm), and yield per plant (8.05 Vs 11.03 g). Such genotypes hold promise for developing high yielding and drought tolerant (low CID) genotypes through hybridization.

Key words: *Glycine max* (L.) Merrill, water use efficiency, CID, diversity analysis

Introduction

Soybean is cultivated in India during *kharif* mostly as rainfed crop [1]. Therefore, it suffers from frequent incidences of drought, which in severe cases leads to death of the plants resulting in immense yield losses. Growing of soybean in India starts with the onset of monsoon i.e. in the second fortnight of June. Usual withdrawal of monsoon occurs in the month of September which coincide with the pod filling stage of soybean. Analysis of rainfall and evapo-transpiration data shows that there is recurrent drought during the month of September that affects pod and seed formation of soybean resulting in its lower yield [2]. Usually, during the month of September, the crop survives on the soil moisture reserved (residual moisture) from the heavy rains of July and August. For such conditions, breeding crop for increased water use efficiency (WUE) has been shown to be the most potent strategy for increasing yield [3]. However, measuring WUE is a highly tedious and nearly prohibitive job particularly in a large breeding population. Carbon Isotope Discrimination (CID) technique is an alternative to measuring WUE and largely applicable in C3 species of plants including soybean [4]. Usefulness of CID for assessing WUE in soybean has already been reported [5]. The present investigation was an attempt for the first time in India in soybean to screen a set of genotypes for WUE through CID technique, and analyze their morpho-physiological characteristics.

Materials and methods

A set of 91 soybean accessions comprising released varieties, advanced breeding lines, exotic and indigenous collections were grown in the experimental field of Division of Genetics, IARI, New Delhi following augmented design [6] using 6 checks (PS 416, PS1024, DS 9814, SL444, EC472183 and DS 9712) in a 5 m single-row plot during *kharif* 2010. Agronomic practices recommended for soybean were followed to raise a good crop. For assessing the water use efficiency (WUE), Carbon Isotope Discrimination (CID) method suggested by Farquhar *et al* [7] was followed. Leaf samples of sixty days old plants under well watered conditions were collected from ten randomly tagged plants in each accession. The leaf samples from five plants were bulked together to make two replications for each accessions. The leaf samples were dried in a hot-air oven at 80°C temperature for 72 hours. The dried leaves

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were ground to fine powder using mortar and pestle. Around 1.0 mg of dried leaf sample was 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 carbon isotopic composition of plant samples (d13cp) was determined with an analytical precision of less than 0.1%. Carbon isotope discrimination (A13C) was computed as per Farquhar *et al.* [7], assuming the isotopic composition of atmospheric air (d13Ca) to be-8%.

D13C (%) = [*d*13Ca – *d*13Cp]/ [1 + *d*13Cp/1000].

All stable isotope measurements were made at the National Facility for Stable Isotope Studies, Department of Crop Physiology, UAS, Bangalore.

Data was also collected on specific leaf weight (SLW), 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 weight was taken as the ratio of leaf dry weight of leaf piece of one square cm area. For leaf ash content leaves were dried in the oven at 70^{o}C 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^{o}C temperature for 2 hours. Variability in the ten quantitative traits was observed in terms of mean, minimum, maximum, range and percentage of coefficient of variability (CV). Correlations among the traits were measured in terms of Pearson Correlation Coefficient and test of significance was done at 5% as well as 1% level of significance with (n-2) degrees of freedom. The accessions with complete data on all the 10 quantitative traits were subjected to hierarchical classificatory analysis using SPSS package. The Euclidean distance was used as a measure of dissimilarity among the accessions and Ward's method was chosen to classify the genotypes and for drawing the dendrogram.

Results and discussion

Variability for water use efficiency (CID or Δ^{13}C)

In the present study, the WUE of 91 genotypes was assessed in terms of CID values (Table 1). The CID values of the accessions ranged from 19.95 to 22.91% (Fig. 1) and, the coefficient of variation for CID was

Table 1. Morphological variability for ten quantitative traits among the 91 diverse accessions of soybean.

	SLA (mg/cm^2)	Leaf ash (Mg/g leaf	CID (%)	Days to 50%	Days to maturity	Plant height	Branches/ plant	Pods/ plant	Seeds/ pod	Seed yield/ plant(g)
N	91.00	89.00	91.00	91.00	89.00	91.00	91.00	91.00	91.00	91.00
Mean	0.0047	0.096	21.691	45.33	107.29	68.48	3.99	61.98	2.15	8.14
Minimum	0.0013	0.073	19.950	37.00	101.00	28.80	1.20	25.60	1.60	1.60
Maximum	0.0083	0.139	22.919	55.00	120.00	122.20	8.60	108.36	3.00	23.80
CV (%)	24.03	11.53	3.36	9.43	3.99	25.23	29.47	33.23	11.18	50.42

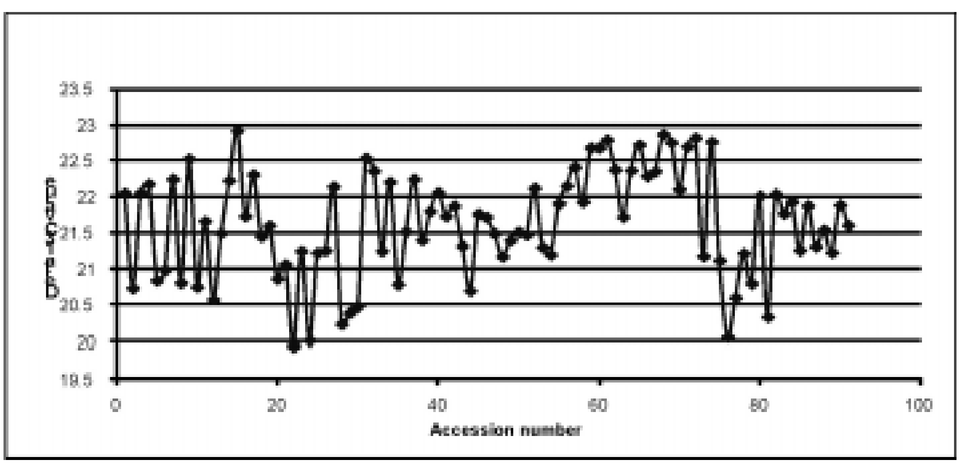

Fig. 1. Variability for CID in 91 genotypes of soybean

3.36%. Genetic variation in WUE has been reported in many field crops, including soybean [6]. High WUE of a genotype can contribute to crop productivity under drought. 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 [8]. In the use of CID as an indirect evidence of WUE, the genotypes which registered lower values of $\Delta^{13}C$ are more water use efficient than the genotypes with higher $\Delta^{13}C$ values. The range of $\Delta^{13}C$ values observed in this experiment is slightly lower than that reported in sugar beet (17.66 to 22.96%) [9]. The CID values are negatively associated with the trait of WUE [10] i.e. the genotypes with lower CID values are more water use efficient than genotypes with higher CID values. Accordingly, genotype DS9813 with lowest CID value (19.95 %) was identified as the one with high WUE, while UPSL 309 with highest CID (22.91%) was noted as the genotype with least WUE. Such genotypes will be suitable for mapping the gene / QTL (Quantitative Trait Loci) for water use efficiency by developing mapping population through hybridization. In total, 7 genotypes were identified as having high WUE (CID < 20.5 %), 12 were low WUE (CID > 22.5 %), and rest were as medium WUE (CID from 20.6 to 22.4 %).

Variability in morphological traits

The tested genotypes were analyzed for variability in the ten quantitative traits in terms of mean, range and coefficient of variation (CV %) and, are summarized in Table 1. The coefficient of variability 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 content (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 is the key for success in plant breeding. Presence of variability in the tested genotypes for different traits including WUE indicates scope of genetic improvement through appropriate breeding approaches.

Correlations among the traits

The correlations among the traits (linear relationship) was determined using Pearson correlation coefficient and the test of significance under the null hypothesis (H_o: r = 0) was done at 5% and 1% level of significance using two tailed test (Table 2). The trait CID (WUE) had non-significant negative correlation with seven out of the nine traits studied. It had significant negative correlation only with maturity (–0.241**). It indicated that with the increase of maturity duration, the WUE of the genotype goes down. Therefore, developing genotypes with shorter maturity duration should get priority to make it water use efficient. Similar results were reported by other workers in soybean [11] as well as in other crops [12]. WUE (as measured through CID) is influenced by

Table 2. Correlation between ten different quantitative traits among 87 diverse accessions of soybean

	SLA (mg/cm^2)	Leaf ash (Mg/g leaf	CID (%)	Days to 50%	Days to maturity	Plant height	Branches/ plant	Pods/ plant	Seeds/ pod	Seed yield/ plant(g)
SLA	1									
Leaf ash (Mg/g leaf dry wt.)	0.005	1								
WUE	-0.032	-0.109	1							
Days to 50% flowering	0.146	0.154	-0.18	1						
Days to maturity	0.308**	0.142	-0.241**	0.273**	1					
Plant height	-0.288**	0.033	-0.063	0.431**	0.186	1				
Branches/pl	-0.238**	-0.029	0.019	0.001	-0.06	0.316**	1			
Pods/pl	-0.24**	-0.041	-0.118	0.025	0.049	0.293**	0.556**	1		
Seeds/ pod	-0.063	-0.057	-0.077	-0.147	0.044	0.124	0.043	-0.001	1	
Seed yield/pl	-0.158	-0.052	0.099	-0.157	-0.127	-0.001	0.402**	0.661**	0.072	1

******Correlation is significant at the 0.01 level (2-tailed).
*****Correlation is significant at the 0.05 level (2-tailed).

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 [13].Thus, every factor which influences both photosynthetic capacity and stomatal conductance processes will also influence WUE. The early maturing genotypes have a faster growth rate and hence a higher photosynthesis capacity giving it an edge over others.

The seed yield per plant was found to be significantly correlated with number of branches (0.402) and pods per plant (0.661). The specific leaf area showed negative correlation with six characters out of nine. However, it was significant for plant height (–0.288), number of branches (–0.238), number of pods (–0.240). Contrary to this a highly significant positive correlation was observed between specific leaf area and days to maturity (0.308). Leaf ash content, similar to seeds per pod did not show any significant association with any other trait studied. Taller genotypes were found to be late flowering (r=0.431) and late maturing (r=0.273), heavy branching (r=0.316) with profuse podding (r = 0.293) but with low specific leaf area (r=–0.288). .

In an effort to find cheap and efficient alternatives to CID, which is a costly technique, specific leaf area (SLA) of the genotypes has been carefully studied. The SLA is an indirect measurement of leaf expansion. Higher SLA represents larger surface area for transpiration and hence more the SLA, less water use efficient the genotype be. Wright *et al*. [14] demonstrated in groundnut that SLA can be used as a rapid alternative to CID in order to estimate genetic variability for WUE. In the present study, the variation in specific leaf area was found to be very high (CV 24%), however, no significant correlation was found between the specific leaf area and CID.

Leaf ash content has also been tried as an alternative to CID technique. Mian *et al*. [5] reported a significant negative correlation (r = –0.40) between water use efficiency (WUE) and leaf ash content in soybean. Masle *et al*. [15] reported a negative association between WUE and leaf ash content across a range of C3 species. In the present study, variability for leaf ash content among the genotypes was low (CV 11%) and, no significant correlation was found between the leaf ash content and CID. It thus indicated that barring cost, CID is the most suitable screening technique for WUE in soybean.

Wright [16] suggested that the positive association between WUE and total biomass yield in a drought environment indicates the possibility of improvement of the WUE of a crop plant resulting in superior yield performance provided a high HI is maintained. Soybean cultivar 'Young' exhibited considerably higher WUE (4.4 g DW) than did 'PI416937' (3.7 g DW) in greenhouse conditions [6]. In another greenhouse study, soybean cultivar 'Jackson' accumulated more biomass and total nitrogen than 'PI416937' with similar transpiration losses [17]. These studies provide evidence that crop WUE can be improved by selecting for genotypic mean yield.

Diversity analysis

The analysis grouped the genotypes into 10 distinct clusters (Fig. 2) each with varying number of genotypes. The cluster means are summarized in Table 3.

Cluster I having 11 genotypes was characterized by the highest seed yield per plant (15.58 g) and branches (5.16). Heavily branched genotypes showed very high podding per plant (85.89), medium days to flowering (44.91), very early maturing (105.45) and medium tall genotypes (75.22 cm) showed very high CID value (21.69%). Genotypes showed low values of specific leaf area (0.04513) and leaf ash content (0.093 mg per gram of leaf dry weight). Cluster II was characterized by the highest CID values (22.43‰) and the lowest days to flower (39.83 days) among all the 10 clusters. The genotypes had shorter genotypes (63.5 cm), medium maturity plants (107.67) with profuse branching (4.47 per plant) and poding (83.7 pods per plant) with high seed yield per plant (11.03 g). Cluster III with only five genotypes was characterized by the highest leaf ash content with medium value of CID and low specific leaf area. Late flowering (48.40 days) and late maturing (110.80 days), taller plant height (78.24cm) with medium number of branches (4.02) and pods (63.71 per plant). The genotypes showed poor performance in seed yield (7.92g per plant). The genotypes of cluster IV had a very high value of specific leaf area (0.00612), very late flowering (50.75 days), late maturity (117.11 days), very tall plants (94.70 cm) with high number of branches (4.90) and very large number of pods (79.9). However, the genotypes had medium number of seeds per pod (2.15) with poor seed yielding capacity (8.05g per plant). Genotypes showed the lowest CID values (20.765 %) along with very low leaf ash content (0.095g per g of dry leaf). Cluster V included 6 genotypes, which were very poor in seed yield in spite of profuse podding (91.59) and branching (4.97). Although the genotypes flowered very late (50.50), yet matured very early

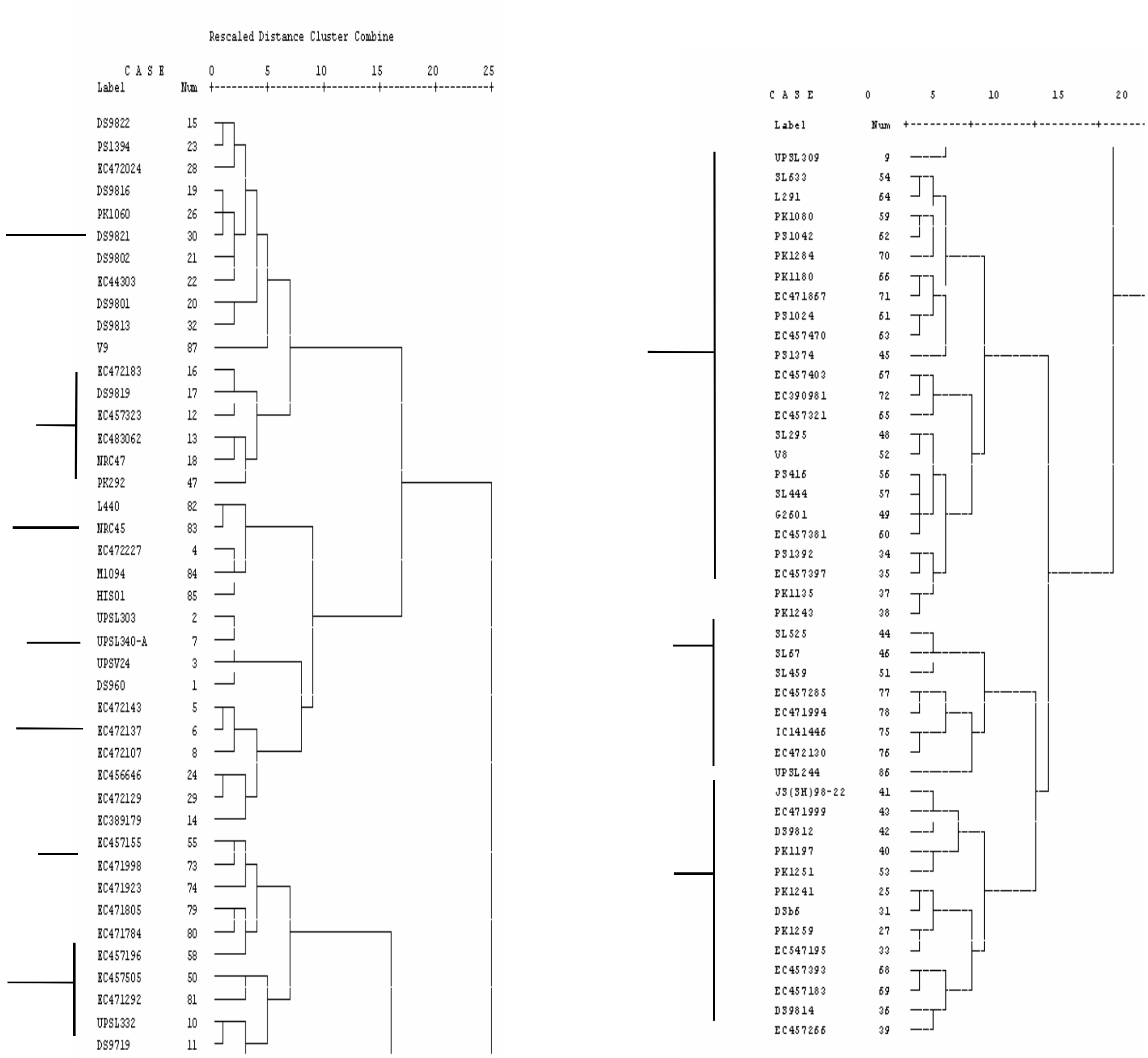

Fig. 2. Dendrogram based on Ward's linkage and Euclidian distance showing grouping of 87 diverse genotypes into ten distinct groups

(106.5). Average value for specific leaf area (0.003810), leaf ash (0.093) and CID (21.01%) for the said cluster ranged from very low to low. Cluster VI has the highest average value of specific leaf area (0.006203) and the lowest for days to maturity (104.83), plant height (55.83 cm), average no. of pods per plant (35.37) and average no. of seeds per pod (1.83). The cluster VI has a very high CID value of (22.25%) and very poor in seed yield (5.17 g per plant). Late flowering (47.80) and late maturing (116.6) genotypes were placed in cluster VII. The genotypes were thinly branched (2.52) with poor poding (41.88) and having medium number of seeds per pod (2.0).Genotypes showed very low value of leaf ash content (0.092) but very high value of specific leaf area (0.0062) and medium CID value (21.76%). Cluster VIII with 23 genotypes was the biggest among all the clusters. The desirable features of this cluster were early flowering (42.12 days), early maturity (105.44 days) shorter plants with medium number of branches per plant. However, the genotypes pertaining to this cluster were very poor in poding (50.91 pods per plant) as well as seed yield (6 g per plant). The cluster had a medium CID value (21.77%) but a very low value of leaf ash content (0.093 mg per gram of leaf dry weight). Cluster IX having 8 genotypes is similar to cluster VIII in all traits except that it had the very tall genotypes (88.33 cm), medium days to flowering (46.63) with medium number of seeds/pod (2.25). Genotypes of the cluster X had the highest average number of seeds per pod (2.41) among all clusters.

Table 3. Cluster means of ten qualitative characters

Cluster No.	No. of geno-	Specific leaf type (mg/cm^2)	Leaf ash area	CID % (Mg/g leaf dry wt.)	Days to 50%	Days to maturity flowering	Plant height	Branches/ plant (cm)	Pods/ plant	Seed/ pod	Seed/ plant (g)
I	11	0.004513	0.093	21.69787	44.91	105.45	75.22	5.16	85.89	2.09	15.58
II	6	0.005029	0.096	22.43391	39.83	107.67	63.50	4.47	83.70	2.30	11.03
III	5	0.004045	0.122	21.43666	48.40	110.80	78.24	4.02	63.71	2.13	7.92
IV	4	0.006121	0.095	20.76521	50.75	117.25	94.70	4.90	79.90	2.15	8.05
V	6	0.003810	0.093	21.01184	50.50	106.50	87.88	4.97	91.59	2.03	6.61
VI	6	0.006203	0.098	22.25385	48.67	104.83	55.83	2.77	35.37	1.83	5.17
VII	5	0.006200	0.092	21.76864	47.80	116.60	62.04	2.52	41.88	2.00	5.00
VIII	23	0.004833	0.093	21.77157	42.12	105.44	58.27	4.10	50.91	2.09	6.00
IX	8	0.003594	0.091	22.20341	46.63	105.88	88.33	3.08	52.05	2.25	5.85
X	13	0.004541	0.097	21.12027	44.65	106.81	58.18	3.55	58.38	2.41	8.91
minimum		0.003594	0.091125	20.76521	39.83	104.83	55.83	2.52	35.37	1.83	5.00
maximum		0.006203	0.122400	22.43391	50.75	117.25	94.70	5.16	91.59	2.41	15.58

The cluster IV contained genotypes with lowest CID values (20.76‰) but had low yield potential, whereas genotypes in cluster II exhibited highest mean CID values with medium yield potential. The highest mean yield was registered by the genotypes in cluster 1 which had medium CID values (21.69‰). The low CID (high WUE) is a conservative trait in terms of efficiency of water use and crop growth rate. In the situation of soil water deficit, low CID genotypes tend to grow slower than high CID genotypes, resulting in lower total biomass production and grain yield [18-20].

The genotypes in Cluster I and V are quite contrasting in most of the characters like CID value (20.76 Vs 22.43 ‰), days to 50% flowering (50.75 Vs 39.43), days to maturity (117.25 Vs 107.67), plant height (94.70 Vs 63.50 cm), and yield per plant (8.05 Vs 11.03 g). Therefore, such genotypes can be used in breeding programme to develop high yielding and drought tolerant (having low CID) genotypes.

While promising results have been obtained, still there are many difficulties regarding the implementation of irrigation techniques in under varying environmental conditions for various crops. Therefore, a deep understanding of the physiological basis for improved WUE in drought sensitive plants is of paramount importance.

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