

Isotope discrimination technique ($\Delta^{13}\text{C}$): A possible selection criteria for drought tolerance in Indian mustard (*Brassica juncea* L.).

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Abstract

Carbon isotope discrimination has emerged as an accurate and time averaged surrogate for water use efficiency among C3 species. Based on this approach, we determined the genetic variability in WUE among a set of 320 germplasm accession of Indian mustard for drought prone areas. The germplasm accessions were raised in an augmented design at National Research Centre on Rapeseed-Mustard, Sewar, Bharatpur (Rajasthan), India during the *rabi* season (October. to March) of 2004-05. The observations on $\Delta^{13}\text{C}$, dry matter accumulation, seed yield, SLA and SPAD (SCMR) values were recorded. $\Delta^{13}\text{C}$ was estimated using Isotope Ratio Mass Spectrometry. The range for $\Delta^{13}\text{C}$ was 18.03- 22.86 per mill, SLA 55.0 – 178.6, dry matter per plant 24.2 – 74.6 g, SPAD value 22.95 – 50.1 and seed yield 6.53 – 21.4 g/plant. The leaf carbon isotope discrimination values ($\Delta^{13}\text{C}$) showed a significant genetic variability and recorded a strong relationship with yield among these accessions suggesting the possibility of adopting $\Delta^{13}\text{C}$ as a selection criterion. Regression analysis revealed up to 71% of the variability in yield could be explained by $\Delta^{13}\text{C}$ in these accessions of mustard. To ascertain this further 40 germplasm accessions with high $\Delta^{13}\text{C}$ and 40 accessions with low $\Delta^{13}\text{C}$ were compared for yield and yield attributes. The low $\Delta^{13}\text{C}$ group showed a significant higher average yield and total biomass when compared with the high $\Delta^{13}\text{C}$ group. Furthermore, the fact that a strong correlation exists between $\Delta^{13}\text{C}$ and yield and no significant differences in test weight and harvest index suggest that WUE is the major determinant of total biomass and yield. The best genotypes having lowest $\Delta^{13}\text{C}$ (BPR 552-9, BPR 349-1, BPR 537- 10, BPR 552-5 and RLM 619) would be utilized in the breeding program. The study revealed for the first time that WUE of these Indian mustard accessions was under the control of photosynthetic capacity and the large variability in this traits can be successfully exploited for further crop improvement.

Key words: water use efficiency (WUE), $\Delta^{13}\text{C}$, Specific leaf area (SLA), Isotope Ratio Mass Spectrometry

Introduction

Among the oilseeds, rapeseed-mustard group of crops occupies prominent position in India as well as in the world. During 2005-06, the rapeseed-mustard crop had worldwide production of 46.65 mt. from an area of 27.17 mha. with an average productivity of 1720kg/ha. Of which, India's share in production and area were about 8.60mt.and 7.2mha., respectively. The yield per hectare of rapeseed-mustard in India (902 kg) was just 56 per cent of the world's average. As maturity duration of Indian cultivars is only 4-5 months compared to almost 10 months in other rapeseed-mustard growing countries like Canada and European countries, the per day productivity of Indian cultivars is quite at par with the world average. Considering the extensive yield losses caused due to drought stress, especially in the tropical world, the major global research focus has been to develop suitable strategies to mitigate the stress effects. The occurrence and severity of drought stress is highly unpredictable and is compounded by the diverse nature of soil physical characteristics, hence rendering drought research highly complicated. Due to these reasons as well as the variations in the extent and mode of response of crop plants to drought stress, progress made to sustain productivity under drought stress has been rather slow. However several traits, physiological process relevant for adaptation to drought have been characterized and validated. Opinion accruing from the literature clearly suggests that mitigating the stress effects would be possible only through introducing relevant 'stress tolerance traits' into an agronomically elite background. Among several traits that impart drought tolerance, WUE, the amount of dry matter produced per unit amount of water transpired, seems to be most relevant. As per Passioura's growth and yield model (1986, 1996), WUE is an important yield-determining factor. Improving water use efficiency (WUE) would reduce the water requirement for a specific yield potential and thus can help save considerable amount of irrigation water. Further, an improvement in WUE can significantly enhance total biomass production as well as yield at a given level of soil water availability.

Though exploitable genetic variability in WUE was documented almost a century ago (Briggs and Shantz, 1913), significant progress in assessing genetic variability in WUE was achieved after the establishment of the physiological links between carbon isotope discrimination ($\Delta^{13}\text{C}$) and WUE (Farquhar and Richards, 1984 ; Farquhar et al., 1989). Several container and field experiments have validated that $\Delta^{13}\text{C}$ is a surrogate of WUE in many crop species (Condon et al., 1987, in *Triticum aestivum* L. and *T. turgidum* L. durum ; Read et al., 1991, in *Agrropyron desertorum* ; Ismail and Hall, 1992, in *Vigna unguiculata* L. Walp ; White et al., 1996 in *Glycine max* (L.) Merr ; Udayakumar et al., 1998 a ; Shshshayee et al., 2003, in several field crops and perennial species).

In the present investigation improved germplasm of Indian mustard for drought prone areas were evaluated for WUE utilizing carbon isotope discrimination technique to identify appropriate donors with high WUE for utilization in the breeding program.

Material and Methods

The experiment was conducted in augmented design during the *rabi* season of 2004-2005 using 320 germplasm including checks Varuna, Kranti and Rohini. The checks were used in each plot in between the breeding lines. Besides 39 released varieties 281 germplasm were also used in the experiment. Each germplasm / breeding line was sown in four row, each of 5 m long. The row-to-row and plant-to-plant distances were kept as 30 cm and 10 cm respectively. The recommended doses of fertilizer (NPK: 80:40:40 kg/ha.) were given to the crop. The half of the recommended dosage of nitrogen and full dose of phosphorus and potassium were applied at the time of sowing and top dressed the remaining half of the N just after first irrigation in the form of urea. The recommended two irrigations were given to the crop at bolting and flowering stage. The thinning and weeding were done at 15 days after the sowing.

The measurement obtained from the SPAD chlorophyll meter (SPAD-502, Minolta Corp., Ramsey, NJ) is based on the differences between light attenuation at 430 nm (the peak wavelength for chlorophyll a and b) and that at 750 nm (near infra red) with no transmittance. Thus the SPAD chlorophyll meter reading (SCMR) represents the chlorophyll concentration in the leaf. The SCMR were made on 70 days old plants in the field trials. The leaves were sampled from the nodal positions three, four and five (fully matured leaves) below the apex on the main axis of three randomly selected plants. Other plant parts (leaves, stem, siliqua and roots) were oven dried at 80°C for 48 hours before determining the plant dry weight. After recording the SCMR, the leaves were processed for the measurement of SLA. The leaf area of the 3 fully matured leaves were measured using a LICOR-3100 leaf area meter after which the leaves were oven-dried at 80°C for at least 48 hours before determining the leaf dry weight. The SLA was calculated as the ration of leaf area to leaf dry weight.

The dried leaf material from each harvest was ground to pass through a 100µm sieve. Carbon isotopic composition in the ground leaf samples was measured by ratio mass spectrometry at the Department of Crop Physiology, University of Agricultural Sciences, GKVK Campus, Bangalore (India).

Results

Owing to the complex city of yield under stress and the multigenic inheritance pattern of yield, further selection for yield may not be remunerative (Araus, 2002) Therefore, breeding for important physiological traits that determine growth and productivity under water limited condition has been projected as the only plausible approach for achieving sustainable productivity under water limited condition.

Table 1. Genotypic variation in $\Delta^{13}\text{C}$, seed yield, test weight, total dry matter, SCMR and harvest index among 320 genotypes of *B.juncea* L.

	$\Delta^{13}\text{C}$	Seed yield	Test wt.	TDM	SCMR	H.I
Mean	20.82	10.7	4.77	39.9	35.9	27.1
Minimum	18.03	6.53	3.35	24.2	27.9	20.1
Maximum	22.86	21.4	5.59	74.6	51.2	35.4
Std. Deviation	0.87	2.13	0.54	8.64	11.9	4.49
C.D.(p=0.05)						
Within check	0.65	1.95	0.46	6.15	2.35	2.57
Within block	2.05	5.02	1.45	19.45	7.43	8.14
Between block	2.36	5.80	1.67	22.47	8.58	9.41
Check-variety	1.80	4.41	1.27	17.1	6.53	7.16

From this context water use efficiency, total water mining associated with roots and partitioning of biomass to the economically important parts (Passioura, 1986) are most relevant traits that deserve exploitation. To assess the genetic variability in these parameters, a large scale screening traits was undertaken with 320 germplasm accessions were screened under irrigated condition in the field.

A significant genetic variability was noticed (table 1). The plant dry matter varied from 24.2 to 74.6g/plant representing a significant variability. Similarly yield varied from 6.53 to 21.4 g/plant. Further a significant relationship between plant dry weight and yield was noticed (fig 1) indicating a possibility of increasing the productivity of mustard through enhancing total biomass. Though considerable variability was noticed in harvest index. The study revealed that a larger variation in biomass was the major factor determined yield among these accessions. This clearly implied the relevance of breeding for improved biomass. It is also evident that efforts to improve the partitioning of biomass to the economically important parts

An attempt was made in this investigation to assess the variability in $\Delta^{13}\text{C}$ among the 320 germplasm accession of mustard. Carbon isotope discrimination values ranged from 18.03 to 22.86 permit representing a significant genetic variability (table 1). A significant relationship between $\Delta^{13}\text{C}$ and yield illustrated in fig. 2 emphasized the fact that $\Delta^{13}\text{C}$ can be used as an effective selection parameter for enhancing the productivity in mustard. The regression analysis revealed up to 71% of the variability in yield can be explained by WUE in these accessions of mustard.

To examine this further the germplasm accession were segregated into two groups based on $\Delta^{13}\text{C}$. The top 40 and bottom 40 accessions were compared for yield and yield attributing parameters. The results are illustrated in fig. 3. The low $\Delta^{13}\text{C}$ group showed a significantly higher average yield and total biomass when compared with the high $\Delta^{13}\text{C}$ group. It was interesting to note that no significant differences in test wt. and harvest index were seen between these categories of genotypes. The results demonstrate that a higher photosynthetic capacity that controlled WUE among mustard accession resulted in

higher biomass therefore yield.

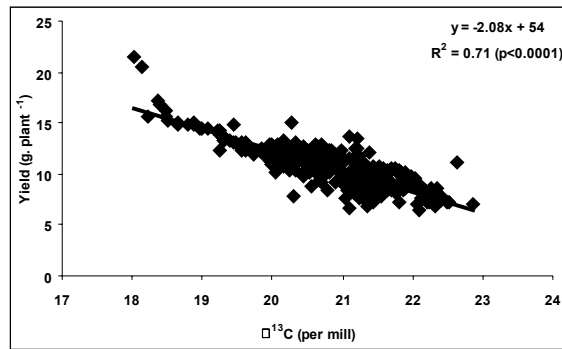


Fig.1 Relationship between seed yield (g.plant⁻¹) and carbon discrimination ($\Delta^{13}\text{C}$) among 320 genotypes of *B. juncea* L.

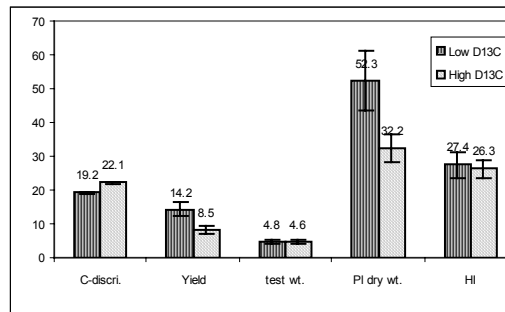


Fig. 2. Relationship between different characters studied in 320 genotypes of *B. juncea* L.

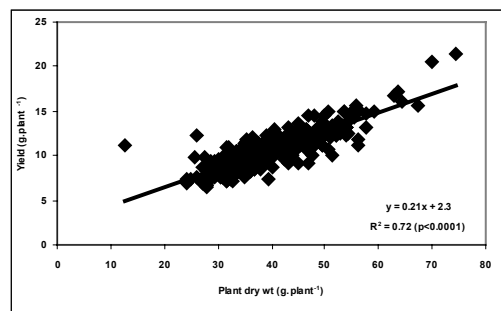


Fig.3 Relationship between seed yield (g.plant⁻¹) and Plant dry weight(g/plant) among 320 genotypes of *B. juncea* L.

Discussion

A significant relationship between plant dry weight and yield indicating a possibility of increasing the productivity of mustard through enhancing total biomass. As per the frame work proposed by Passioura, water use efficiency is an important determinant of growth. Despite it's importance large scale breeding traits have not yet been under taken, owing mainly to the difficulty involved in the determination of genetic variability for water use efficiency. Tremendous impact to these efforts was provided with the discovery of the physiological link between WUE and carbon isotope discrimination (Farquhar and Richards, 1984; Farquhar et al., 1989). This technique has been extensively used in several breeding program to enhance crop productivity under water limited condition (Richards et al., 2002; Sheshashayee et al., 2003; Condon et al., 2004; Impa et al., 2005).

A significant relationship between $\Delta^{13}\text{C}$ and yield illustrated in fig. 2 emphasized the fact that $\Delta^{13}\text{C}$ can be used as an effective selection parameter for enhancing the productivity in mustard. The regression analysis revealed up to 71% of the variability in yield can be explained by WUE in these accessions of mustard. Such relationship between $\Delta^{13}\text{C}$ and yield is normally encountered when the photosynthetic capacity of the chloroplast are high (Lloyd and Farquhar, 1993 ; Richards et al., 2002 ; Udayakumar et al., 1988 ; Sheshashayee et al., 2003). Similar report of chloroplast capacity controlling the differences in WUE was reported for groundnut by Rao et al (1995).

Conclusion

This is perhaps the first report of demonstrating the relevance of $\Delta^{13}\text{C}$ in mustard breeding program to improve productivity. Further experiments to confirm these observations and to study the relevance of high WUE under water limited condition are being carried out.

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