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Variation in carbon isotope discrimination and its relationship with harvest index in the reference collection of chickpea germplasm.

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Abstract

Chickpea is largely grown rainfed and terminal drought is a major constraint to its productivity. Transpiration efficiency (TE) is an important component of grain yield under drought. The reference collection of chickpea germplasm was used to assess the extent of variation in TE through carbon isotope ratio ($\delta^{13}\text{C}$), its association with yield and yield components. The field trials were conducted both with optimum and without irrigations in a Vertisol during 2008-09 and 2009-10 post-rainy seasons. Leaves collected at 63 days after sowing were analyzed for $\delta^{13}\text{C}$. Drought stress reduced shoot biomass by 36% in 2008-09 and 39% in 2009-10 but the yield reduced by 23% in 2008-09 and 0% in 2009-10. Poor partitioning in the optimally irrigated crop caused this zero yield reduction. Mean $\delta^{13}\text{C}$ was low under optimal irrigation and was relatively high under drought stress and the range of genetic variation among the reference collection accessions (-2.9) was the highest under drought stress. Accessions away by two-folds of standard error of difference from the mean towards the lowest were 20 and highest were 21. Most low $\delta^{13}\text{C}$ accessions were early flowering (40-50 d), with moderate shoot biomass, high seed yields and high harvest indices (HI). $\delta^{13}\text{C}$ was positively correlated with final shoot biomass of the accessions under drought stress in 2009-10. $\delta^{13}\text{C}$ was positively correlated with the seed yield under drought stress in both the years and under optimally irrigation in 2008-09 but more tightly and negatively with HI. Among the yield components, $\delta^{13}\text{C}$ was closely associated with pod numbers per unit area in the drought stressed environments in both the years and in 2008-9 under optimal irrigation. $\delta^{13}\text{C}$ was positively correlated with 100 seed weight only under drought stress in both the seasons. Path coefficient analysis showed $\delta^{13}\text{C}$ had no direct contribution to grain yield but indirectly positively through shoot biomass at maturity and heavily negatively through HI. The highest contribution of HI or shoot biomass was seen in the maturity group of accessions that experienced the right terminal drought stress rather than the imposed

drought stress or optimal irrigation. In the absence of direct contribution of $\delta^{13}\text{C}$ to drought tolerance, direct selection for both final shoot biomass and HI is suggested.

Introduction

Globally, chickpea (*Cicer arietinum L.*) is the third most important grain legume crop, widely grown across the Mediterranean basin, East Africa, Indian-subcontinent, Americas and Australia. Approximately 90% of world's chickpea is grown under rainfed conditions (Kumar and Abbo, 2001) where terminal drought is one of the major constraints limiting the productivity. The global demand for chickpea in 2050 is projected to be 18.3 Mt compared to a 2010 supply of 9.4 Mt and with the low income food deficit countries suffering the widest supply-demand gap (IMPACT model developed by IFPRI; Rosegrant et al. 2008). Large part of this deficit needs to be met through breeding for drought tolerance and yield stability. A trait-based breeding approach is being increasingly emphasized over yield-based breeding for realizing better stability as grain yield show low heritability and heavily influenced by high G×E interactions (Ludlow and Muchow 1990). Such trait-based breeding approaches are particularly needed to improve crop productivity under water limited conditions for which a thorough understanding of plant characteristics that confer yield advantages is necessary. Enhancing growth rates and productivity under water limited conditions can be possible only when most relevant drought tolerance alleles are transgressed into a single genetic background.

In conditions where water is limited, a water-based analytical framework has been used for a long time to decompose yield construction into several components, i.e. a better water mining abilities associated with the root system, superior water use efficiency or transpiration efficiency (TE) for biomass production and good partitioning ability for increased realization of economic yield (Passioura 1977). The availability of large and significant genetic variability for some of the root related traits (Kashiwagi et al. 2005), knowledge on their genetic control (Kashiwagi et al. 2008) and the identification of robust QTLs associated with these traits (Varshney et al. 2009) is considered as a major accomplishment towards breeding for enhanced drought tolerance and encourages searches for variations for other traits related to drought tolerance. More recent result also clearly indicates the importance of temporal pattern of plant water use,

in particular the capacity of tolerant genotypes to use a larger portion of their water budget during the post-anthesis period, therefore supporting a better seed setting (Zaman-Allah et al., 2011a).

Understanding TE and the extent of variation available for TE in chickpea is considered next in priority. Higher plant biomass productivity per unit water absorption is understandably a characteristic of importance for crop improvement under water limited conditions. However, the measurement of TE through gravimetric means is considered cumbersome and expensive. Although, an alternate protocol had been shown to make it possible (Krishnamurthy et al., 2007), still, carbon isotope discrimination is used as a surrogate for TE as it offers the convenience of storage of test tissue and a small sample requirement. Plants are known to vary in their discrimination against heavy isotope of carbon during photosynthesis under less intercellular CO₂ concentration, leading to a higher ¹³C concentration in low transpiration efficient genotypes than in the high transpiration efficient genotypes (Farquhar et al. 1989). Relatively early stomatal closure is, thus, shown to prevent further water loss and improve TE. Carbon isotope discrimination has been well established as a surrogate for water use efficiency (WUE) (Udayakumar et al., 1998; Sheshshayee et al., 2003; Impa et al., 2005). An advantage of this TE characterization method is the ability to handle larger population size using long stored, if required, test samples. In several legumes such as bean (White, 1993; Wright and Redden, 1995), cowpea (Ismail and Hall, 1992; Ismail et al., 1994), groundnut (Hubick et al., 1986; Wright et al., 1994), lentil (Matus et al., 1995), soybean (White et al., 1995; Uday Kumar et al., 1996; Tobita et al., 2007), and in chickpea (Kashiwagi et al. 2006b) $\Delta^{13}\text{C}$ was found to be correlated with TE. But the lack of such relationship between $\Delta^{13}\text{C}$ and TE was also shown, in groundnut transgenic events (Devi et al., 2011) or in three legume species grown well-watered (Turner et al. 2007), or a weak relationship was found in recombinant inbred lines of groundnut (Krishnamurthy et al., 2007), indicating that there are specific weather and soil moisture conditions where the contribution of $\Delta^{13}\text{C}$ becomes apparent. There were also indications from these works that there can be direct as well indirect effects of the isotope discrimination on yield performance and special attention is required to understand such effects (Mohankumar et al. 2011; Khazaie et al. 2011)

A reference collection of chickpea germplasm based on molecular diversity data of global composite collection (3000 accessions at 50 microsatellite loci) (Upadhyaya et al. 2006) has been developed at ICRISAT (Upadhyaya et al. 2008). True to its molecular diversity, the reference collection of chickpea had seen to exhibit enormous diversity for reactions towards high temperature (Krishnamurthy et al. 2011a) and salinity (Krishnamurthy et al. 2011b). Therefore, the objective of this work was 1. to identify the extent of variation available for carbon isotope discrimination and the extreme contrast sources in the reference collection of chickpea germplasm both under terminal drought stress and optimally irrigated conditions, 2. understand the type of relationship of carbon isotope discrimination with seed yield and yield components.

2. Materials and methods

2.1. Crop management

The cultivated accessions of reference collection of chickpea germplasm (n=280) were evaluated under field during the post-rainy seasons of 2008-09 and 2009-10 on a Vertisol (fine montmorillonitic isohyperthermic typic pallustert) at ICRISAT, Patancheru (17° 30' N; 78° 16' E; altitude 549 m) in peninsular India. The soil depth of the fields used in 2008-09 was ≥ 1.2 m and in 2009-10 was 1.1 m. and these soils retained about 230 mm (2008-09) and 205 mm (2009-10) of plant available water in the 120-cm (maximum rooting depth) soil profile. The fields used were solarized using polythene mulch during the preceding summer to sanitize the field, particularly to eradicate *Fusarium oxysporum* wilt causing fungi as chickpea was often grown in these fields. Later the field was kept fallow and Glyphosate (Roundup®) herbicide was applied prior to land preparation only during 2008-09.

The fields were prepared into broad bed and furrows with 1.2 m wide beds flanked by 0.3 m furrows for all the experiments. Surface application and incorporation of 18 kg N ha⁻¹ and 20 kg P ha⁻¹ as di-ammonium phosphate was carried out in all the experiments. The plot size was 4.0 m x 2 rows in both the seasons. The experiments were conducted with two irrigation levels as main plot treatments (1. Drought stressed: nonirrigated except for a post-sowing irrigation and 2. Irrigated: optimally irrigated depending on the need) in a 14 x 20

alpha design (280 accessions) with three replications. Seeds were treated with 0.5% Benlate® (E.I. DuPont India Ltd., Gurgaon, India) + Thiram® (Sudhama Chemicals Pvt. Ltd. Gujarat, India) mixture in all the three seasons. Both the experiments were hand planted at the first opportunity after the cessation of the rains on 31 Oct 2008 and 31 Oct 2009 in rows 30-cm apart with 10cm between plants at 3-5 cm depth with two seeds per hill which was later thinned to one. During all the seasons, the fields were inoculated with Rhizobium strain IC 59 using liquid inoculation method (Brockwell, 1982). A 50 mm irrigation through perforated pipes was applied the next day to ensure complete emergence. Successive irrigations, to the irrigated treatments were through furrow irrigation. Intensive protection against pod borer (*Helicoverpa armigera*) was provided and the plots were kept weed free by manual weeding.

2.2. Leaf sampling for carbon isotope measurements

At 63 days after sowing, ten new fully expanded leaf from major primary branches of different plants were collected, dried below 80°C in a convection oven for 3-days and were fine-powdered in a vibrating mill. Duplicated subsamples were applied to a dynamic flash combustion type element analyzer (EA-1112, Carlo Erba, Milan, Italy). An adequate quantity of CO₂ gas separated and purified by the EA was introduced to an isotope ratio mass spectrometer (Delta XP Plus, ThermoFinnigan, Hamburg, Germany) to estimate the ratio of the isotopic composition (¹³CO₂/¹²CO₂), which is expressed as δ¹³C.

By regular observation, the date when 50% or more of the plants in a plot flowered was recorded as 50% flowering time of the plot and when 80% of the pods in a plot were dried was recorded as the time of maturity for each plot.

2.3. Soil moisture measurements

Neutron moisture meter access tubes were installed in four spots planted with two drought tolerant (ICC 867 and ICC 14778) and two drought sensitive accessions (ICC 6263 and ICC 8058) (Krishnamurthy et al. 2010) in an adjacent broad bed in each replication and treatment

during both years. Neutron moisture meter (Depth Moisture Gauge, Model 3332, Troxler Electronic Laboratories Inc., NC., USA) readings at soil depths of 15cm increments up to a depth of 120 cm were made before and after each irrigation as well as matching it at about 10 d intervals or close by. The Troxler soil moisture observations were corrected using a calibration curve developed for each depth separately using the data collected gravimetrically across the season. Moisture content of the surface soil (0-15cm) was measured only gravimetrically. The water held in each soil horizon of 15cm depth was summed up to 1.2 m and presented.

2.4. Final harvest

At physiological maturity, plant aerial parts were harvested from an area of 2.7 m² in 2008/09 and 2009-10 in each plot, dried to constant weight in hot air dryers at 45°C, and total shoot dry weights were recorded. Grain weights were recorded after threshing. Harvest index (%) was calculated as $100 \times (\text{Seed yield}/\text{total shoot biomass at maturity})$. Plants from 0.5×0.75m area were used for the estimation of pod number and seed number per m² and their weights. 100 seed weight was estimated from these seed weight and numbers.

2.5. Statistical analysis

The replication-wise values of yield and yield components were used for statistical analysis of each environment using ReML considering genotypes as random. Variance components due to genotypes (σ_g^2) and error (σ_e^2) and their standard errors were determined. Environment wise best linear unbiased predictors (BLUPs) for the mini core accessions and others were calculated. Heritability was estimated as $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$. The significance of σ_g^2 was tested against its standard error.

For the pooled analysis, homogeneity of variance was tested using Bartlett's test of homogeneity (Bartlett 1937). In pooled analysis (environment) was considered as a fixed effect and the genotypes as random. The variance due to (G) (σ_g^2) and (G) × (E) interaction (σ_{gE}^2) and their standard error were determined. The significance of the fixed effect of the year was assessed using the Wald statistic that asymptotically follows a χ^2 distribution. The means derived were used for the correlations and the path coefficient analysis.

Path analysis in this case was sought to dissect the correlations into different components of direct and indirect effects due to large number of independent variables that are expected to contribute to the dependant variable, the grain yield.

3. Results

3.1. Weather during crop growth season

The rains received prior to the cropping season (842 mm in 2008-09 and 902 mm 2009-10) of chickpea was higher than normal and ensured complete charging of the soil profile before sowing. The weather during the crop growing seasons varied from the long term in the time of cessation of seasonal rains. During 2008-09 between 15 and 30 days after sowing there were incidences of rain ranging from 10 to 15 mm per week totaling 26 mm. Such early rain incidences during 2009-10 were between 9 to 19 days totaling 44 mm (Fig 1). The overcast and the drizzle during these rain incidences delayed the onset of drought under drought stressed crop. There was another rain (39 mm) at 75 days after sowing during 2009-10 but this was too late for most of the drought stressed accessions to get benefitted with and for most irrigated accessions to suffer excessive vegetative growth and lodging. Overall the minimum temperatures were higher in general and particularly during the critical third and fourth week of Dec (flowering and early-podding season of the adapted germplasm) and maximum temperatures were lower during 2009-10. The daily evaporation that was around 3 to 4 mm increased to 5 and beyond after 82 in 2008-09 and 91 days after sowing in 2009-10.

3.2. Changes in soil moisture across growing season

Broadly, the pattern and the rate of soil moisture depletion did not vary between the two seasons under drought stressed condition except that the 2008-09 season started with greater available soil moisture and this difference between the years was maintained till 30 days after sowing (Fig 2). Once again the available soil water in the soil profile was the lowest in 2009-10 under drought stress between 60 and 70 days after sowing. At complete maturity under drought stress the available soil water left unutilized was about the same and less than 50 mm. Most of this water was found below 60 cm soil profile (data not shown). Irrigations at the early stages of the season raised again the total

available soil water to initial levels whereas as the season advanced irrigations did not charge the profile fully, although the water available was sufficient to fully support plant growth in the irrigated treatment (Fig 2).

3.3. Effect of terminal drought on phenology of chickpea germplasm

The overall means for each irrigation treatment across years had shown that drought stress advanced the days to 50% flowering by 3 to 4 days and the days to maturity by 17 days (Table 1). The range of predicted means for days to 50% flowering did not show this effect like the means. Drought stress usually enhanced flowering. By the time, early maturing accessions started flowering, the drought stress was not intense enough to see this enhancing effect and therefore the early maturing accessions flowered at the same time both under nonirrigated and irrigated conditions. Whereas, in the very late accessions, an intense drought effect brought about a contrary result by not allowing the drought stressed plants to desiccate and dry without flowering. The accessions tested ranged and varied widely with a maximum difference of about 32 days for days to 50% flowering and about 36 days for days to maturity under drought stress. But these differences for days to maturity were markedly narrow when optimally irrigated; 17 days in 2008-09 and 8 days in 2009-10.

3.4. Effect of terminal drought on yield and yield components of chickpea germplasm

Mean shoot biomass produced in 2009-10 was marginally more than in 2008-09 in both the moisture environments. Drought stress reduced shoot biomass by 36% in 2008-09 and 39% in 2009-10 (Table 1). The accessions varied two-fold in shoot biomass in both the soil moisture environments and years. But the mean seed yield was similar in both the years in the drought stressed environment while it was substantially higher in 2008-09 in the irrigated treatment. The accessions ranged more than 4-fold variation under drought stressed and three-fold under irrigated environment. The harvest index, in general, was poor in 2009-10. Under drought stressed condition it was 37.7 compared to 45.5 and under irrigated condition it was 22.7 compared to 39.4 percent. Drought stress increased harvest index. There was a huge variation among accessions for the harvest index. Pod

number m^{-2} performance was very close to the seed yield performance. Mean 100 seed weight was similar across years and drought stress improved mean 100 seed weight. The accessions tested exhibited a large variation for seed size both under drought stress and optimally irrigated environments.

3.5. Effect of terminal drought on carbon isotope ratio ($\delta^{13}C$) of chickpea germplasm
Mean $\delta^{13}C$ was very close between the two seasons under optimally irrigated conditions whereas it was -27.0‰ in 2008-09 and -25.8‰ in 2009-10 under drought stressed condition. Drought stress increased this value. And this increase was substantial during 2009-10 when the drought stress was severe at the time of sampling due to less water holding capacity of the soil (Fig 3). The range of genotypic variation was the highest under drought stress during 2009-10 (-2.9‰) followed by drought stress during 2008-09 (-2.6‰). This range of ratio was the least under optimum irrigation during 2009-10 (-1.2‰). Lowest isotope ratio was found under optimally irrigated 2009-10 as well as the narrowest range among the accessions. The highest isotopic ratio was found under drought stress in the 2009-10 season with the highest range of $\delta^{13}C$ values. Accessions above (high $\delta^{13}C$) and below (low $\delta^{13}C$) two-folds of standard error of difference were identified and listed in table 2. The low $\delta^{13}C$ accessions were twenty and the high $\delta^{13}C$ ones were twenty one. Most of the low $\delta^{13}C$ accessions were 40-50 day flowering time group with moderate shoot biomass productivity and high seed yields with high harvest indices. They are largely small seeded.

3.6. Relationship of $\delta^{13}C$ with phenology and yield components

Correlations

$\delta^{13}C$ was not correlated with either the days to 50% flowering or maturity with the exception of 2009-10 drought stressed treatment. This relationship was closest in the drought stressed crop of 2009-10 when drought stress was relatively severe. $\delta^{13}C$ was also positively correlated with shoot biomass at maturity of the accessions closely in the drought stressed treatment during 2009-10. $\delta^{13}C$ was correlated at a 5% probability or closer with the seed yield under drought stressed in both the years and under optimally irrigated only during 2008-09. These negative correlations of $\delta^{13}C$ were even closer with

HI in this soil moisture regimes and seasons. Among the yield components, $\delta^{13}\text{C}$ was closely associated with pod numbers per unit area in the drought stressed environments in both the years and in year 2008-9 under optimally irrigated environment. $\delta^{13}\text{C}$ was positively correlated with 100 seed weight only under drought stress in both the seasons. When similar irrigation environments of the two year were pooled, these correlations appeared again under drought stress environment alone with the phenology traits and all the other yield and yield components but under irrigated conditions $\delta^{13}\text{C}$ contributed to shoot biomass, HI and pod number m^{-2} .

Path analysis

Path coefficients provide absolute measures of contribution of variation, both direct and indirect, thereby assisting to assign appropriate significance and choice for selection for use in breeding programs. The path coefficients showed that the direct effect of $\delta^{13}\text{C}$ on seed yield was about nil in either of the season or soil moisture environments, individually or pooled. Similarly the indirect contribution of $\delta^{13}\text{C}$ through phenology and the yield components such as pod number m^{-2} and 100 seed weight was also almost nil. The two components through which the contributions were the total shoot biomass that contributed positively and the HI contributing negatively (Table 4). However, $\delta^{13}\text{C}$ had major indirect contribution to seed yield primarily through harvest index. In general, $\delta^{13}\text{C}$ contributed more under drought stressed condition than under optimally irrigated through HI followed by total shoot biomass (Table 4).

3.7. Contribution of $\delta^{13}\text{C}$ to grain yield in groups of varying phenology

The contribution of $\delta^{13}\text{C}$ may change across phenologically different groups of accessions that started flowering from 35 days to 70days after sowing. As the accessions tested fell in a large range of flowering time groups i.e. from terminal drought escapes to the ones that face extreme level of drought stress intensities with delays in flowering. Therefore the accessions were segregated into 4 groups (i.e. group 1 = accessions that has taken <41 days for 50% flowering, group 2 =between 41-50 days, group 3 = between 51-60 days and group 4 = accessions that has taken >60 days for 50% flowering) and the correlations and the path analysis was performed. Group 1 accessions were early and

likely to have escaped drought. Phenology of group 2 accessions were the best adapted for this region with a possible exposure to mild to moderate drought stress. Group 3 accessions were exposed high intensities of drought whereas the group 4 accessions did suffer intense drought and heat as seen through their poor range of harvest indices and greater shoot biomass. Again in all these 4 groups the path coefficients showed no significant direct effect of $\delta^{13}\text{C}$ on seed yield or through any other phenology or yield component traits other than total shoot biomass and HI. Therefore, the magnitude of indirect contributions (coefficients) is presented in Fig 3. The salient features are:

1. Irrespective of soil moisture, year or the phenological group the major contribution of $\delta^{13}\text{C}$ to seed yield came through the harvest index.
2. The highest negative contribution of harvest index was seen in the 41-50 day flowering group under drought stress but such contribution was highest in the 51-60 group when optimally irrigated.
3. These contributions were relatively larger during 2009-10 when the drought stress was severe.
4. The indirect contribution through the shoot biomass or harvest index decreased with increase in phenology under drought stressed condition whereas it increased under optimally irrigated condition
5. It is clear that the relationship between $\delta^{13}\text{C}$ and the seed yield was close in the two medium phenology groups of accessions. In the early phenology group, as these accessions would have escaped drought, and the late phenology group, as these accessions would have suffered severer stress levels, the genotypic discrimination was low or nil.

There was a close positive relationship between the path coefficients of shoot biomass and the harvest index (Fig 5). This association can be seen only among the second and the third group of genotypes. Such a relationship can not be seen when the first group or the fourth group of genotypes are included in this relationship. This close relationship was indicative of the existence of a matching tradeoff between the total shoot biomass and the partitioning and a negative linkage between the two traits i.e. shoot biomass and HI.

Discussion

Variation and possible use of $\delta^{13}\text{C}$

In this study $\delta^{13}\text{C}$ is seen to contribute indirectly to chickpea yields under drought stress. But this relation was loose and occasional. Here, 20 accessions of the reference collection (n=280) were identified to possess the lowest $\delta^{13}\text{C}$. Twelve of them were reported to rank as the top drought tolerant accessions (based on their stress yields), in a previous study that dealt with the minicore chickpea germplasm that shares 211 accessions with the reference collection (Krishnamurthy et al. 2010). Reports from a large number of studies, involving collections of bread wheat, durum wheat and barley grown in rain-fed and irrigated environments in Australia and elsewhere, show a positive or neutral relationships between grain yield and $\Delta^{13}\text{C}$ frequently (as reviewed by Condon et al., 2004) confirming that $\delta^{13}\text{C}$ can reflect in yield. Also there is a clear relationship of less negative $\delta^{13}\text{C}$ with both higher shoot and root dry weights in a wild barley germplasm (Robinson et al. 2000) as well as with yield and straw biomass of glaucousness variant isolines of cultivated barley (Febrero et al 1998) indicating $\delta^{13}\text{C}$'s contribution to other traits known to confer yield advantages

With the occasional exceptions in this study, the low $\delta^{13}\text{C}$ accessions were relatively early in flowering and maturity as seen in Arabidopsis (McKay et al. 2003). These accessions also had a not-so-high shoot biomass, a high harvest index, greater pod numbers per unit area and smaller seeds. Similar observations were also made in wheat (Farquhar and Richards 1984; Ehdaie et al. 1991) and groundnut (Rao et al. 1993; Farquhar and Richards, 1984; Ehdaie *et al.*, 1991). Blum (2005) raised a concern that indiscriminate selection for higher WUE (or lower $\delta^{13}\text{C}$) with the assumption that it equates with improved drought resistance or improved yield under stress might bring about serious negative consequences for the yield potential of the crop. High WUE is largely a function of reduced water use rather than a net improvement in plant production or biochemistry of assimilation. Therefore in selection programs it may constitute a marker for reduced water use commonly achieved via moderated growth, reduced leaf area, and short growth duration. If low water use is the breeders target it is highly probable that selection for low water use can be achieved by directly selecting for these

simple plant traits, without measuring WUE (Blum 2005). Similarly, a direct use of component traits that contribute to TE was suggested to increase the effective use of available water through the growing season to ultimately maximize the growth and yield of the crop rather than the TE (Sinclair 2012). Such a suggestion arose as TE was hard to resolve for use in breeding programs due to its high dependency upon both physiological and environmental variables. The results of this study also confirm the crop responses described by Blum (2005) of the low and high WUE selections. However, it has also been recently demonstrated in a large set of sorghum germplasm, where water use and WUE were assessed gravimetrically in lysimeters and over long periods of time, that WUE bore no significant relationship with total plant water use, indicating that high WUE could be reconciled with high water use (Blum et al., 2011). Here, the fact that a large proportion of these high WUE accessions produced consistently the best drought yields under drought (across three years) in severely water limiting environments (Krishnamurthy et al. 2010) overrules these concerns of poor water use or less biomass production as far as the receding soil moisture environments of Patancheru is concerned. Selection for $\delta^{13}\text{C}$ seems to offer also the subtle balance of fitting the phenotype to the soil moisture environment. Moreover selection for both $\delta^{13}\text{C}$ and high photosynthesis had been shown to be achievable and a clear 10% yield advantage had been demonstrated in wheat by following a dual selection approach (Rebetzke et al. 2002). This had been practiced by first selecting lines on stomatal conductance, thereby eliminating those with poor photosynthetic performance and selecting for water use efficiency in a second step. Also there is a need to look for variations that are non-physiological in nature but known to influence $\Delta^{13}\text{C}$ and yield as leaf glaucousness had been shown to influence the $\Delta^{13}\text{C}$, biomass and yield in cultivated barley (Febrero et al. 1998) and early flowering and maturity to influence the $\Delta^{13}\text{C}$ in Arabidopsis (McKay et al. 2003).

$\delta^{13}\text{C}$ and its relationship with yield and other yield components

The greatest challenge to using $\Delta^{13}\text{C}$ in breeding for greater agronomic water use efficiency is the high level of inconsistency observed in the relationship between $\Delta^{13}\text{C}$ and yield (Condon et al. 2004). Such conflicts of $\delta^{13}\text{C}$'s contribution to yield seem to be influenced by soil moisture environments (Rowland and Lamb 2005; Tardieu 2011). $\delta^{13}\text{C}$

is a product of differences that are largely influenced by the variations in stomatal conductance. When soil moisture levels are decreased, a common plant response is simultaneous decreases in photosynthesis, transpiration and leaf conductance (Farquhar et al. 1989). If the leaf conductance decreases at a faster rate under stress than the photosynthetic dependence on intercellular CO₂ concentration, then intercellular CO₂ concentration will decrease. This effect is measurable as a decrease in carbon isotope discrimination. Therefore variable discrimination for carbon isotopes is possible only when the plant leaves were exposed to adequately longer periods of critical water deficits. Development of water deficit is dynamic and growing crops under terminal drought stress need or need not develop drought stress of the required intensity all through the growing period. Even in crops that are grown under receding soil moisture conditions, at least the early growth stages in winter season when the vapor pressure deficits are at the lowest, the appearance of drought stress is relatively minimal to affect the stomatal conductance. However, the stress can develop gradually and transform severe with the extent of delays in maturity (Saxena et al. 2003; Krishnamurthy et al 2010). As a consequence the accessions that flower from 35 to 71 days after sowing experience increasing intensities of drought with the increase in flowering time; the drought intensities vary from drought escape in extra-early and early entries to suffering extreme intensities of drought by the late duration entries. Some accessions in the irrigated treatments were also subjected to longer spells of drought and heat stress at the late grain filling period as a result of their excessive longer duration as in such experiments the decisions to stop the irrigation is taken considering the mean growing period of overall entries of the experiment. As a consequence, though not intended, the accessions in reality complete their life cycle under varying drought intensities and durations. Therefore the exposure of the accessions to drought stress in such experiments can lead to a range of $\delta^{13}\text{C}$'s contribution to yield. Therefore the characteristic of the final response curve of any group of accessions depends upon both the genetic variation in stomatal conductance (Condon et al. 2004) under drought and the quantum of exposure to drought stress. As the absence of either one parameter can lead to the absence of a response curve (Tardieu 2011), the chances of noticing a response curve is one to four. Therefore even an occasional appearance of a response is enough to claim the useful contribution of $\delta^{13}\text{C}$.

It has been clearly shown in this experiment that $\Delta^{13}\text{C}$ contributes to seed yield indirectly, either through the total shoot biomass or harvest index. TE is known to be negatively related to transpiration and positively to harvest index (Blum 2005). The path coefficients had indicated that the extent of positive contribution through shoot biomass brings in a matching quantum of negative contribution through harvest index (Fig 5). Finally the larger contribution of HI is seen on the seed yield as also shown in other crops (Condon et al. 2004). This relationship suggests the existence of a close linkage between $\delta^{13}\text{C}$ -induced shoot biomass and harvest index. Therefore it is quite likely that breeding for $\delta^{13}\text{C}$ involves linkage drags and thus there could be appearance of molecular markers that are common to both $\delta^{13}\text{C}$ and HI or shoot biomass and harvest index. For example, Correlation coefficients calculated using major chunk of the accessions (n= 243 to 254) showed $\delta^{13}\text{C}$ (inversely TE) to be negatively correlated with harvest index (r = 2008-09 drought stressed -0.40, optimally irrigated -0.41, 2009-10 drought stressed -0.64 and optimally irrigated 0.16). Similar relationships were elusive in the too early or too late groups of accessions that were very few and close to 15% of the 280 accessions. These relationships suggest that a direct selection for both high harvest index and total shoot biomass could be more targeted and beneficial than for $\delta^{13}\text{C}$. Similar alternatives of component selection was also suggested for TE as TE is considered to be a difficult trait to resolve in many breeding programs (Sinclair 2012). A case study of physiological changes that occurred in wheat varieties released from 1962 to 1988 by CIMMYT also supports this breeding and selection strategy (Fisher et al. 1988). Here, more recent wheat varieties were said to be substantially high in grain yields under irrigation and these yields were found to be associated with the harvest index but not with greater biomass production (Condon et al. 2004). This collection of CIMMYT wheats had no change in total biomass production despite the more recent wheats having both higher photosynthetic capacity and higher stomatal conductance, together generating substantially higher values of photosynthesis on a leaf area basis.

Conclusions

There was a large range of variation for $\delta^{13}\text{C}$ in the reference collection of chickpea germplasm that was well spread under drought stress than under optimal irrigation.

Drought stress increased $\delta^{13}\text{C}$ values compared to optimal irrigation. $\delta^{13}\text{C}$ was correlated with seed yield more under drought stress and the poor relationship was more due to less exposure to drought stress. $\delta^{13}\text{C}$ contributed to seed yield, indirectly, positively through total shoot biomass and negatively through HI. Selection for lesser $\delta^{13}\text{C}$ or for greater TE balanced the shoot biomass production and HI to produce the best seed yields. The concern that selection for higher TE would automatically mean selections for lesser shoot biomass is valid, and two layers of selection one for higher $\delta^{13}\text{C}$ and the other for shoot biomass are required when dealing with this trait.

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Table 1

Trial means, range of best linear unbiased predicted means of accessions (BLUPs) and analysis of variance of the 280 accessions of reference set of chickpea germplasm for phenology, shoot biomass at maturity, seed yield and a few yield components in the field experiments during postrainy seasons of 2008-09 and 2009-10 under both drought-stressed and optimally-irrigated environments.

Season/Environment	Trial mean	Range of predicted means	S.Ed	σ^2_g (SE)	Heritability (h^2)
2008-09, Drought stressed					
$\Delta^{13}C$	-27.0	-28.0 – -25.4	0.392	0.2825 (0.0334)	0.726
Days to 50% flowering	51.1	38.8 – 70.9	1.77	35.53 (3.12)	0.966
Days to maturity	98.2	83.2 – 119.7	2.08	38.78 (3.48)	0.946
Shoot biomass (kg ha ⁻¹)	3444	2259 – 4726	501.6	220188 (28546)	0.677
Seed yield (kg ha ⁻¹)	1557	510 – 2250	802.9	91544 (10202)	0.777
Harvest index (%)	45.5	11.4 – 60.5	3.05	80.1 (7.21)	0.943
Pods (number m ⁻²)	1358	512 – 2332	255.3	118937 (12762)	0.805
100 seed weight (g)	16.3	8.8 – 36.2	1.13	34.95 (2.996)	0.988
2008-09, Optimally irrigated					
$\Delta^{13}C$	-28.0	-28.9 – -26.6	0.385	0.1916 (0.0252)	0.679
Days to 50% flowering	54.4	40.4 – 69.2	2.31	42.98 (3.84)	0.953
Days to maturity	115.3	109.3 – 125.8	1.95	7.47 (0.82)	0.816
Shoot biomass (kg ha ⁻¹)	5263	3602 – 6977	648.3	447678 (62609)	0.631
Seed yield (kg ha ⁻¹)	2057	1004 – 3029	311.4	138923 (16309)	0.737
Harvest index (%)	39.4	17.3 – 52.8	3.85	48.16 (4.60)	0.894
Pods (number plant ⁻¹)	1966	802 – 3297	451.7	332582 (37146)	0.781
100 seed weight (g)	15.7	7.2 – 44.6	1.14	33.97 (2.92)	0.988
2009-10, Drought stressed					
$\Delta^{13}C$	-25.8	-27.1 – -24.2	0.476	0.4071 (0.0487)	0.739
Days to 50% flowering	48.4	34.8 – 65.7	2.00	37.78 (3.38)	0.948
Days to maturity	95.2	78.7 – 114.7	3.18	82.03 (7.41)	0.940
Shoot biomass (kg ha ⁻¹)	4120	2822 – 5499	430.9	303086 (37854)	0.737
Seed yield (kg ha ⁻¹)	1518	442 – 2314	209.3	134255 (13672)	0.860
Harvest index (%)	37.70	11.3 – 57.0	2.77	132.99 (11.60)	0.973
Pods (number plant ⁻¹)	42.6	21.2 – 71.2	7.50	105.6 (12.3)	0.749
100 seed weight (g)	17.3	9.2 – 44.8	1.31	38.32 (3.32)	0.978
2009-10, Optimally irrigated					
$\Delta^{13}C$	-28.04	-28.6 – -27.4	0.276	0.0860 (0.0137)	0.581
Days to 50% flowering	52.5	35.8 – 64.4	3.05	40.37 (3.88)	0.893
Days to maturity	112.2	108.2 – 116.6	1.46	3.726 (0.451)	0.723
Shoot biomass (kg ha ⁻¹)	6781	3566 – 8926	773.1	1086034 (129241)	0.733
Seed yield (kg ha ⁻¹)	1522	694 – 2311	302.8	136055 (17861)	0.655

Harvest index (%)	22.7	10.9 – 37.8	3.67	38.08 (3.94)	0.828
Pods (number plant ⁻¹)	48.6	28.6 – 86.9	11.82	168.8 (25.4)	0.591
100 seed weight (g)	15.1	8.1 – 33.9	1.55	22.59 (2.02)	0.948

Table 2. Mean carbon isotope ratio of the top (2 standard errors of difference below the grand mean) and bottom (2 standard errors above the grand mean) ranking accessions out of 280 and their days to 50% flowering, days to maturity, shoot biomass (kg ha⁻¹), seed yield (kg ha⁻¹), harvest index (%), pods (number m⁻²), seeds pod⁻¹ and 100 seed weight (g) across two seasons under drought stressed environment. .

S.No.	Accession	$\delta^{13}\text{C}$	Days to 50% flowering	Days to maturity	Shoot biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Harvest index (%)	Pod number m ⁻²	Seed pod ⁻¹	100 seed weight (g)
Low $\delta^{13}\text{C}$										
1	ICC 1422*	-27.59	40.3	86.5	3642	1992	56.3	1612	1.03	17.4
2	ICC 762*	-27.55	65.3	114.4	3456	950	25.7	954	1.20	11.1
3	ICC 15868*	-27.53	50.7	94.5	3077	1490	50.5	1289	1.40	11.1
4	ICC 5221	-27.35	47.0	91.9	3147	1543	51.6	1461	1.15	11.3
5	ICC 5878*	-27.31	43.5	88.0	2878	1452	54.5	1335	1.18	11.0
6	ICC 11279	-27.30	56.7	115.0	2537	389	10.2	758	1.13	9.8
7	ICC 10945*	-27.30	45.3	90.1	3465	1724	51.2	1677	1.14	13.9
8	ICC 8318*	-27.26	38.8	86.6	3367	1824	56.9	1396	0.99	19.3
9	ICC 11498	-27.24	53.1	101.4	3750	1600	43.1	1451	1.24	12.6
10	ICC 1882*	-27.22	42.6	89.1	3532	1905	55.5	1669	1.01	14.0
11	ICC 4918	-27.20	41.4	86.3	3607	1953	56.6	1480	1.02	18.7
12	ICC 1398*	-27.20	40.8	84.8	3503	1968	58.4	1383	1.06	17.9
13	ICC 12916	-27.16	55.0	101.9	3864	1659	43.1	1217	1.28	12.1
14	ICC 10466	-27.15	44.3	94.1	3461	1736	51.7	1874	1.28	10.8
15	ICC 283*	-27.15	42.2	90.4	3328	1817	57.3	1670	1.13	13.5
16	ICC 3946*	-27.15	57.2	103.2	3946	1341	34.1	1486	1.15	11.4
17	ICC 15612	-27.14	43.9	87.3	3616	1714	48.9	1463	1.02	15.5
18	ICC 440	-27.14	54.9	100.2	4052	1821	45.1	1404	1.22	11.7
19	ICC 1098*	-27.11	49.8	94.5	3971	1975	50.8	1716	1.12	13.4
20	ICC 9002*	-27.10	49.3	93.9	3420	1710	51.8	1385	1.27	12.1
High $\delta^{13}\text{C}$										
1	ICC 10500	-25.75	52.7	97.4	4506	1681	37.1	1092	0.98	21.5
2	ICC 10885	-25.74	55.7	104.7	4237	1232	27.0	650	0.88	28.4
3	ICC 3761	-25.74	45.7	90.6	3705	1508	40.9	1504	1.20	10.8
4	ICC 1052*	-25.74	48.3	96.9	3642	1255	34.8	1152	1.37	11.4
5	ICC 12379	-25.74	54.6	105.5	4435	1352	29.6	857	1.01	27.6
6	ICC 11303	-25.74	49.4	100.6	4217	920	21.6	613	1.05	40.5
7	ICC 8752	-25.72	52.5	103.6	4118	1141	27.1	1053	1.08	14.0
8	ICC 6875	-25.71	53.3	99.9	4212	1263	29.4	945	0.99	18.6
9	ICC 13599	-25.64	51.9	98.2	3844	1407	36.0	957	1.03	19.7
10	ICC 8855	-25.63	41.9	90.9	3920	1813	47.8	1427	1.07	17.0
11	ICC 11903	-25.62	53.8	106.9	4128	1274	29.6	979	0.92	26.0
12	ICC 2990*	-25.54	52.6	103.9	3759	1329	36.2	1032	1.01	17.6
13	ICC 8515	-25.45	60.4	112.2	4641	1144	23.0	959	1.07	15.4
14	ICC 7819*	-25.41	50.6	99.8	4123	1497	36.3	1189	1.01	21.1
15	ICC 12028	-25.36	51.0	99.0	4168	1489	35.7	1178	1.05	19.6
16	ICC 9712	-25.28	51.1	96.2	4454	1542	34.0	1391	1.13	14.6
17	ICC 7554	-25.15	51.5	99.5	4049	1300	32.4	953	1.00	21.9
18	ICC 6905	-25.13	54.6	107.8	4690	970	18.9	736	0.93	25.5
19	ICC 6293	-25.08	51.1	100.9	3984	1362	34.2	1140	1.24	11.2
20	ICC 3239	-25.07	55.1	108.8	3445	871	24.7	787	1.02	15.0
21	ICC 10569	-25.01	52.1	100.4	3448	1261	35.9	887	1.25	18.7

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- Entries appear as drought tolerant and highly sensitive ones in Krishnamurthy et al. 2010

Table 3. Correlation coefficients of carbon isotope ratio ($\delta^{13}\text{C}$) with days to 50% flowering, days to maturity, shoot biomass (kg ha^{-1}), seed yield (kg ha^{-1}), harvest index (%), pods (number m^{-2}), seeds pod^{-1} and 100 seed weight (g) across 2008-09 and 2009-10 seasons under both drought stressed and optimally irrigated environment.

Year/Irrigation	Days to 50% flowering	Days to maturity	Shoot biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Harvest index (%)	Pod number m^{-2}	100 seed weight (g)
2008-09, Drought stressed	-0.020	0.101	0.154	-0.190	-0.333	-0.382	0.321
2008-09, Optimally irrigated	0.082	0.117	0.160	-0.222	-0.396	-0.262	0.097
2009-10, Drought stressed	0.403	0.445	0.514	-0.439	-0.628	-0.443	0.301
2009-10, Optimally irrigated	-0.124	-0.021	0.154	-0.007	-0.101	-0.085	0.057
Both years, Drought stressed	0.232	0.343	0.431	-0.378	-0.574	-0.494	0.354
Both Years, Optimally irrigated	0.021	0.096	0.250	-0.184	-0.350	-0.221	0.075
Both years, both irrigations	0.187	0.293	0.437	-0.362	-0.555	-0.455	0.263

Table 4. Path coefficients showing the direct and indirect contributions [through days to 50% flowering, days to maturity, shoot biomass (kg ha⁻¹), harvest index (%), pods (number m⁻²) and 100 seed weight (g)] of carbon isotope ratio ($\delta^{13}\text{C}$) to seed yield during the 2008-09 and 2009-10 seasons under both drought stressed and optimally irrigated environment.

Year/Irrigation	$\delta^{13}\text{C}$	Days to 50% flowering	Days to maturity	Shoot biomass (kg ha ⁻¹)	Harvest index (%)	Pod number m ⁻²	100 seed weight (g)	Seed yield (kg ha ⁻¹)
2008-09, Drought stressed	-0.016	0.000	0.000	0.105	-0.290	0.009	0.002	-0.190
2008-09, Optimally irrigated	0.006	-0.002	0.004	0.118	-0.354	0.012	-0.005	-0.222
2009-10, Drought stressed	-0.020	0.014	0.005	0.301	-0.725	-0.011	-0.002	-0.439
2009-10, Optimally irrigated	0.008	-0.002	0.001	0.082	-0.091	-0.004	-0.001	-0.007
Both years, Drought stressed	-0.041	0.006	0.000	0.268	-0.610	0.001	-0.003	-0.378
Both years, Optimally irrigated	0.000	0.000	0.003	0.161	-0.343	0.000	-0.005	-0.184
Both years, both irrigations	-0.027	0.006	0.017	0.278	-0.619	-0.006	-0.010	-0.362

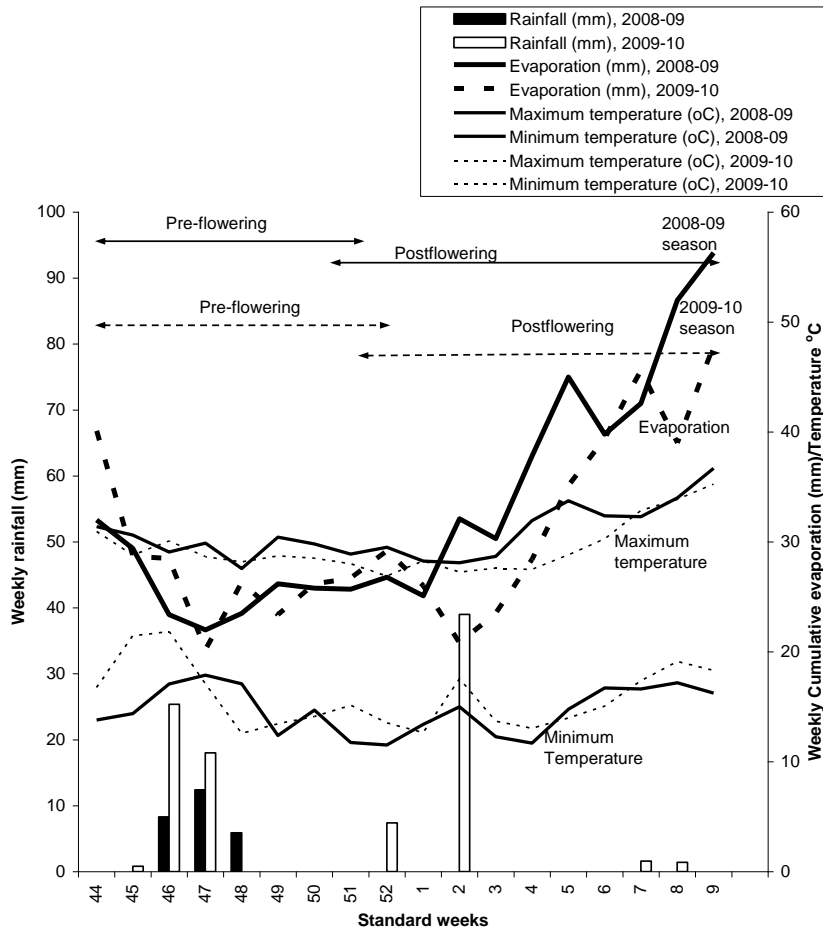


Fig 1. Weather during the cropping seasons of 2008-09 and 2009-10 postrainy season at Patancheru.

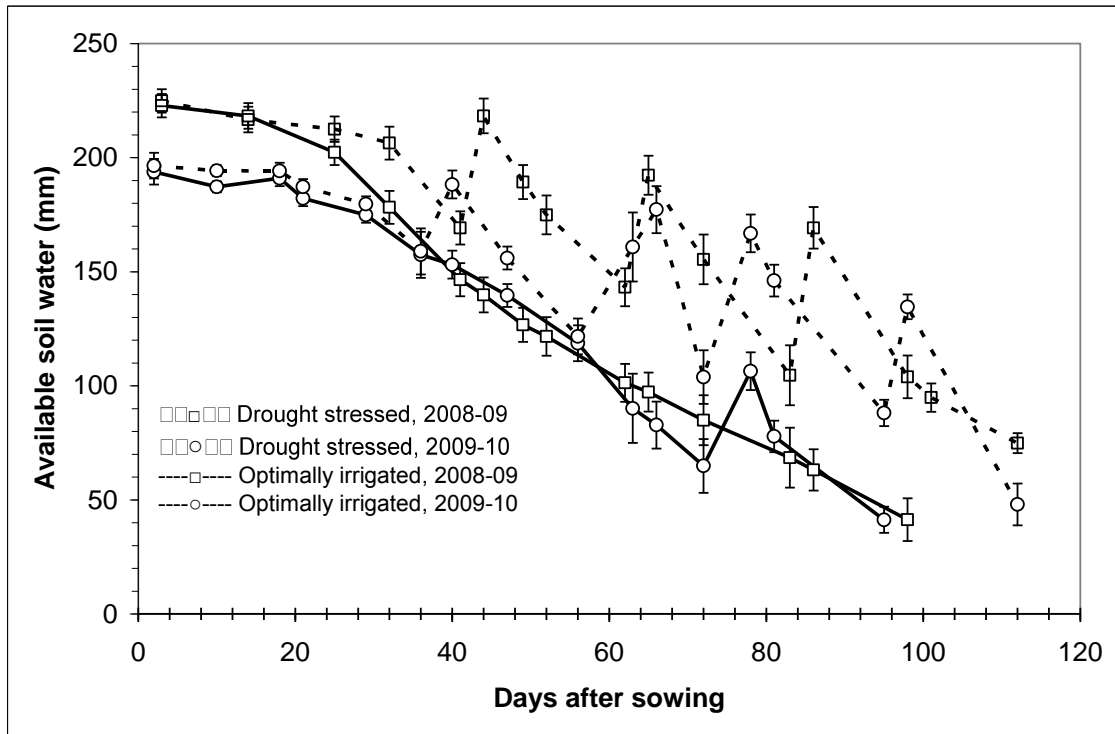


Fig 2. Changes in available soil moisture up to a soil depth of 1.2m across the crop growing seasons of 2008-09 and 2009-10. Vertical bears denote standard error of differences (\pm).

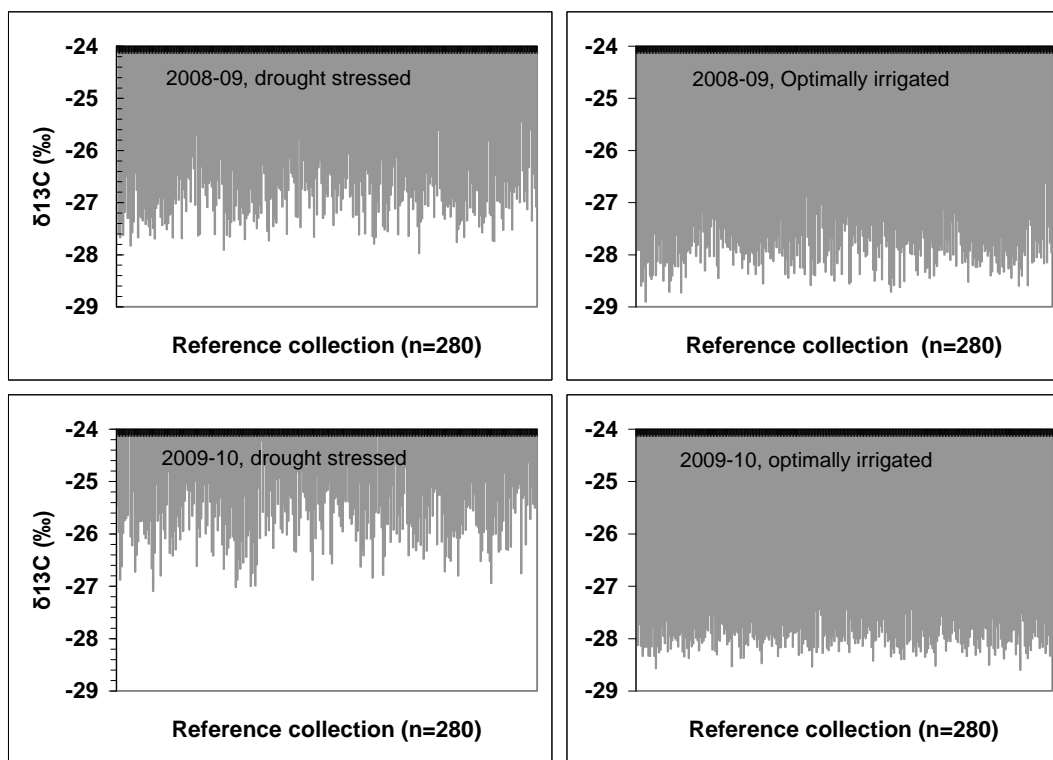


Fig 3. Distribution of carbon isotope ratio (‰) in the reference collection accessions of chickpea germplasm (n=280) under drought stressed and optimally irrigated conditions in two (2008-09 and 2009-10) post-rainy seasons.

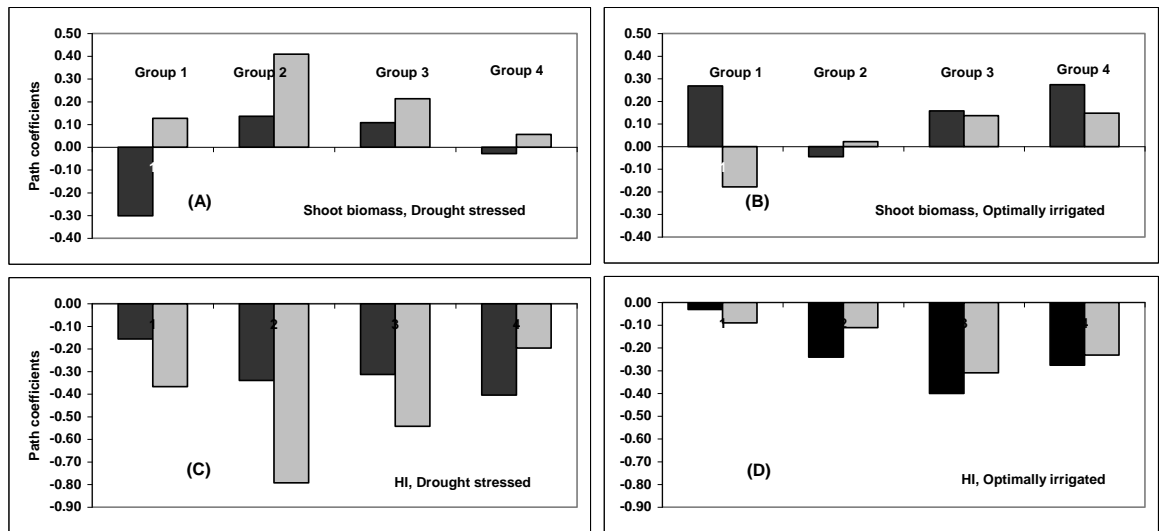


Fig 4. Contribution of carbon isotope ratio to seed yield through (A) shoot biomass in all the four phenological groups under drought stressed condition, (B) optimally irrigated condition and through (C) harvest index in all the phenological groups in the drought stressed condition (D) and optimally irrigated condition. Solid black bars are the contributions during 2008-09 and grey bars are during 2009-10.

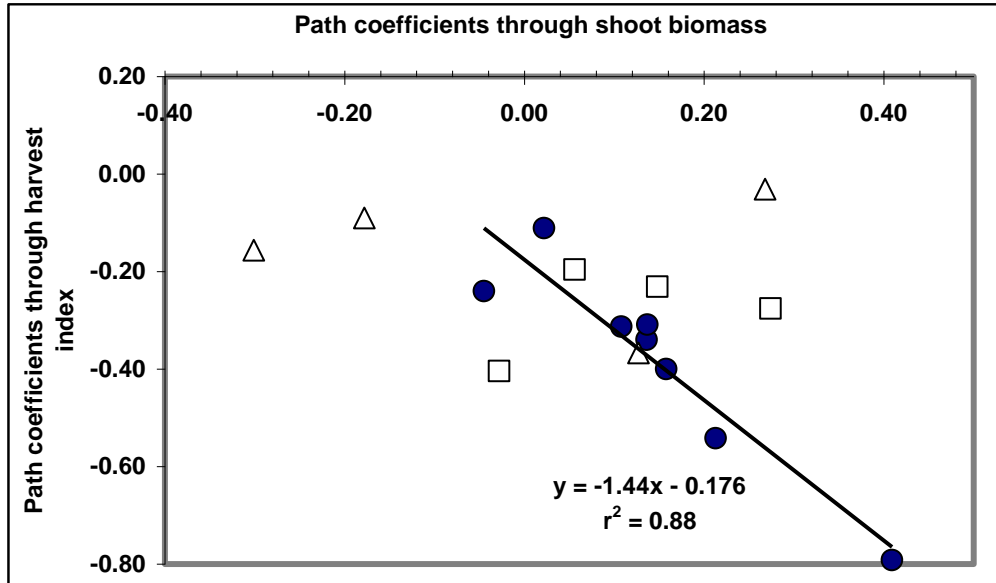


Fig 5. The relationship between the path coefficients of shoot biomass and the harvest index in the first group (open triangle), second and third group (solid rounds) and the fourth group (open triangle). The regression coefficient and the trend line are specific to the second and third group points only.

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