

Characterization and Molecular Mapping of Drought Tolerance in Kabuli Chickpea (*Cicer arietinum* L.)

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Saskatoon

By

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Abstract

Drought is the most common abiotic stress limiting chickpea production in the world. Ninety percent of the world's chickpea is produced in areas relying upon conserved, receding soil moisture, therefore, crop productivity is largely dependent on efficient utilization of available soil moisture. Because of the variability in drought pattern from year to year, trait based selection could have an advantage over selection on the basis of grain yield alone. Trait based breeding, however, requires trait dissection into components. Successful marker identification would facilitate integration of MAS procedures in breeding programs enabling the pyramiding of favourable alleles.

The genetic map produced in this study was based on a population of recombinant inbred lines of a cross of ILC 588 x ILC 3279 containing 52 SSR markers spanned 335 cM of the chickpea genome at an average density of 6.4 cM. A total of 13 genomic regions were shown to be associated with drought tolerance traits. Some of these genomic regions showed pleiotropic effect on multiple traits. This was also supported by the analysis of phenotypic data where these traits were found to be correlated. For example, early flowering and maturity had a strong association with high grain yield. High grain yield was also associated with better partitioning ability between biomass and grain yield, i.e. harvest index. Drought tolerance score (DTS) was associated with various important traits including biomass, early flowering, early maturity.

This study also concluded that chickpea genotypes differed in terms of root length, root length density, root weight density and root length to weight ratio at every 20 cm soil layer up to 100 cm depth in response to water deficits. Consideration of an efficient root system vs. a larger root system is also important, since in this research,

large root systems were offset by low harvest index, presumably due to the lack of assimilate available for grain growth. A restricted root system is important in environments like Western Canada, where crop growth termination is usually required prior to fall frost. This study also reported significant associations of stomatal conductance (g_s) with each of HI, grain yield under drought, drought susceptibility index and drought tolerance score (DTS). Stomatal conductance can also be used to assess plant stress due to drought. Values of g_s less than $250 \text{ mmol m}^{-2}\text{s}^{-1}$ during flowering indicated drought stress under greenhouse conditions. A higher degree of plant stress due to drought was shown by increased stomatal closure at midday ($g_s < 150 \text{ mmol m}^{-2}\text{s}^{-1}$). The study of 157 RILs under natural drought stress during 2005-07 revealed that the 17 RILs which had high grain yield under drought (Group A), also tended to have higher g_s than the 42 RILs that had lower grain yield (Group B). Group A had mean g_s values of $390 \text{ mmol m}^{-2}\text{s}^{-1}$ during the week before flowering, while Group B had mean g_s value of $330 \text{ mmol m}^{-2}\text{s}^{-1}$. Stomatal conductance increased at flowering and then sharply decreased later in the reproductive period, particularly in Group B. These findings were also supported by canopy temperature differential measurements as Group A was also able to maintain lower canopy temperature than Group B, indicating the ability of these plants to maintain adequate transpiration and a cooler canopy under drought stress. This research indicated that g_s and canopy temperature can be used to assess chickpea drought stress and to screen drought tolerant genotypes. This study identified a QTL on LG7 for g_s , QTLs on LG1, LG3 and LG6 associated with canopy temperature differential, as well as QTLs associated with grain yield under drought, HI, DTS, days to flower, days to maturity, reproductive period and plant height. These QTLs identified for traits related to

higher chickpea productivity under drought stress could have important implications for accelerating the process of pyramiding of favourable genes into adapted genotypes and on future marker-assisted breeding for drought prone areas.

Dedicated

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Abbreviations and Acronyms

AFLP – amplified fragment length polymorphism

CDC – Crop Development Center, University of Saskatchewan

CGIAR – Consultative Group on International Agricultural Research

DAF - DNA amplification fingerprint

DSI – Drought susceptibility index

FAO – Food and Agricultural Organization of United Nations

g_s – Stomatal conductance ($\text{mmol m}^{-2}\text{s}^{-1}$)

ICARDA – International Center for Agricultural Research in the Dry Areas

ICRISAT – International Crops Research Institute for the Semi-Arid Tropics

ISSR - inter simple sequence repeat

LG – linkage group

MAS – marker-assisted selection

QTL – quantitative trait loci

RAPD - random amplified polymorphic DNA

RILs – recombinant inbred lines

ROS – reactive oxygen species

SSR – simple sequence repeats

STMS - sequence tagged microsatellite site

Tc-Ta – Canopy temperature differential ($T_{\text{canopy}} - T_{\text{air}}$)

1. Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinating, annual crop with a diploid set of chromosomes ($2n=2x=16$). The estimated genome size of chickpea is 740 Mb (Arumuganathan and Earle, 1991).

Chickpea is an important legume crop in the Semi-Arid Tropics (SAT) and the West Asia and North Africa (WANA) regions and is becoming an important crop in new regions including Australia and North America. Chickpea ranks third in production among the world pulse crops (FAO, 2006) after dry bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.). Currently, it is produced on 10.7 million ha with annual production of 8.2 million tonnes (FAO, 2006). The majority of this area is concentrated in Asia (9.8 million ha) with a production of 7.4 million tonnes followed by Africa (0.4 million ha) with a production of 0.3 million tonnes and the Americas (0.3 million ha) with a production of 0.4 million tonnes (FAO, 2006). Chickpea is a major export commodity in Australia (\$66 million) and North America (\$45 million) during 2005 (FAO, 2006).

Chickpea was introduced to western Canada only recently and production began in the 1990s. Chickpea production in Saskatchewan increased from 2400 ha in 1996 to over 460,000 ha in 2001 (Agriculture Statistics Handbook, 2001, Saskatchewan Agriculture, Food and Rural Revitalization) with more than 90% being concentrated in the Brown (Aridic Haploborolls) and Dark Brown (Typic Borolls) soil zones (Gan and Noble, 2000). However, the area of production decreased substantially in 2002–2004, because of high disease pressure of ascochyta blight and problems associated with late maturity. Chickpea area increased somewhat in 2005 and reached 112,571 ha during 2006 with a production of 137,200 tonnes (Saskatchewan Agriculture and Food, 2006).

Chickpea is an indeterminate crop which can continue to grow for extended periods under cool or wet conditions (Fig. 1.1). The duration of crop growth (commonly less than 100 days) in drier areas in India and the Mediterranean region is cut short by drought and high temperature. Chickpea faces diverse environments in these and other production areas in terms of photoperiod, temperature and precipitation, all of which have a profound effect on growth and development (Khanna-Chopra and Sinha, 1987). The time of sowing and the photoperiod varies among these regions but generally most of the precipitation is received before or during the early crop season and generally the crops matures under progressively declining soil moisture and increasing temperature. In most of the chickpea growing areas, drought is a prominent characteristic which limits seed yield and can even lead to total crop failure. In both Mediterranean and sub-tropical climates, seed filling in chickpea is subject to terminal drought which limits seed yield (Turner *et al.*, 2001).

Due to the increase in global land degradation over the past 50 years (CGIAR, 1995) as a result of agricultural activities and increasing global population, there is pressure on agriculture for increased food production. The potential of higher productivity is greatest in marginal, stressed environments because of its lower productivity in the past as compared to productivity under favorable environments.

Breeding efforts for improvement of drought tolerance in crop plants is primarily based on selection for grain yield under drought stress. Because of the variability in drought pattern from year to year, further progress may not be achieved by selecting

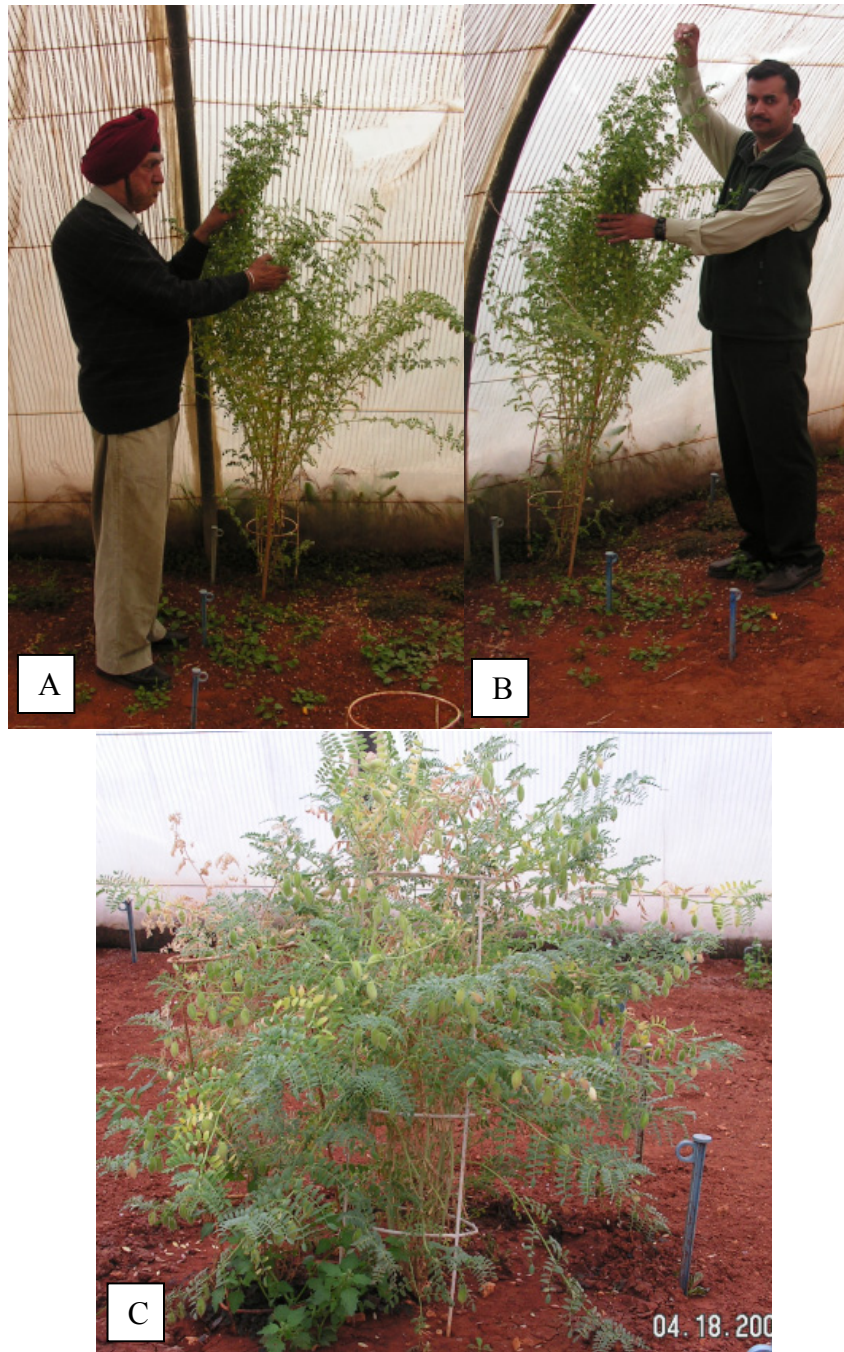


Fig. 1. 1: Chickpea plant (>2 m tall) in a greenhouse (A, B, C) at ICARDA, Aleppo, Syria which grew for over six months during 2006-07 due to its indeterminate growth habit.

solely for grain yield. To overcome the low response to direct selection, substantial efforts have targeted the manipulation of morpho-physiological traits influencing drought resistance through escape, avoidance and / or tolerance mechanisms (Ludlow and Muchow 1990; Blum 1996).

Improving crop production in stress environments is feasible with new technologies and knowledge. A viable solution for yield improvement in crops is the understanding of its physiological and molecular basis. Hence, physiological and molecular based plant breeding could be critical for further progress in improving yield potential and yield stability. Hence, this research had the following objectives.

1. To characterize eight diverse chickpea genotypes for agronomic and physiological parameters related to drought under non-stress and water stress treatments under greenhouse conditions, and identify key traits to use for characterization of an intraspecific population under natural drought stress conditions.
2. To identify QTLs associated with agronomic and physiological parameters of 155 recombinant inbred lines of an intraspecific chickpea population under natural drought stress for future use in marker-assisted selection for higher productivity under drought.

2. Literature Review

2.1 Drought resistance mechanisms

Drought is the most economically important abiotic constraint to crop production in the world (Araus *et al.*, 2002; Boyer, 1982). Drought can be defined as below normal precipitation that limits plant productivity (Kramer and Boyer, 1995). A drought situation can be classified as either terminal or intermittent. During terminal drought, the availability of soil water decreases progressively and this leads to severe drought stress at the later period of crop growth and development. Intermittent drought is the result of finite periods of inadequate rain or irrigation occurring at one or more intervals during the growing seasons and is not necessarily lethal.

Although host-plant tolerance is an important objective in many plant breeding programs, understanding of the physiological mechanisms that contribute to variability in crop performance in drought environments remains limited (Cecereilli and Grando, 1996; Passioura, 1996). Many physiological processes associated with crop growth and developments including photosynthetic CO₂ assimilation, transpiration and stomatal regulation, cell growth, hormonal and enzyme concentration etc. are influenced by water deficits (Hsiao, 1973, Boyer and McPherson, 1975, Begg and Turner, 1976, and Turner and Begg, 1978). There have been many attempts to classify drought-resistant plants (May and Milthorpe, 1962; Parker, 1968; Levitt, 1972; Arnon, 1975). May and Milthorpe (1962) utilized 'drought' as a meteorological term, that is, a period without significant rainfall. They identified three types of drought resistance viz; (a) drought escape; the

ability of a plant to complete its life cycle before serious soil and plant water deficits develop; (b) drought tolerance with high tissue water potential; and (c) drought tolerance with low tissue water potential. According to Levitt (1972, 1980), two main mechanisms by which plants adapt to drought are drought escape and drought resistance. Quisenberry (1982) defined drought resistance as the ability of a plant variety to produce a higher yield than another at a given limiting level of water availability. A simplified conceptual diagram of crop plant adaptation mechanisms in response to decreased water availability is presented in Fig. 2.1. Under drought escape and avoidance mechanisms, the plant balances water uptake and loss to avoid an effect of the stress on tissue water potential. Stress is dealt by the plant outside the plant tissue. If this cannot be achieved and the plant tissue does experience low water potential, then dehydration tolerance mechanisms must respond to ensure plant tissue growth and survival. For agricultural context, drought escape and dehydration avoidance mechanisms are important for productivity. The three primary types of drought resistance mechanisms are described in the following section.

2.1.1 Drought escape

Drought escape can be defined as the ability of a plant to complete its life cycle before a serious plant water deficit develops. Selection for rapid phenological development is a common approach in breeding for drought resistance in crops. Jordan *et al.* (1983), Saeed and Francis (1983), Bidinger *et al.*, (1982), Laing and Fischer (1977) and Saeed *et al.*, (1984) have shown that late maturing genotypes were better adapted to wet conditions,

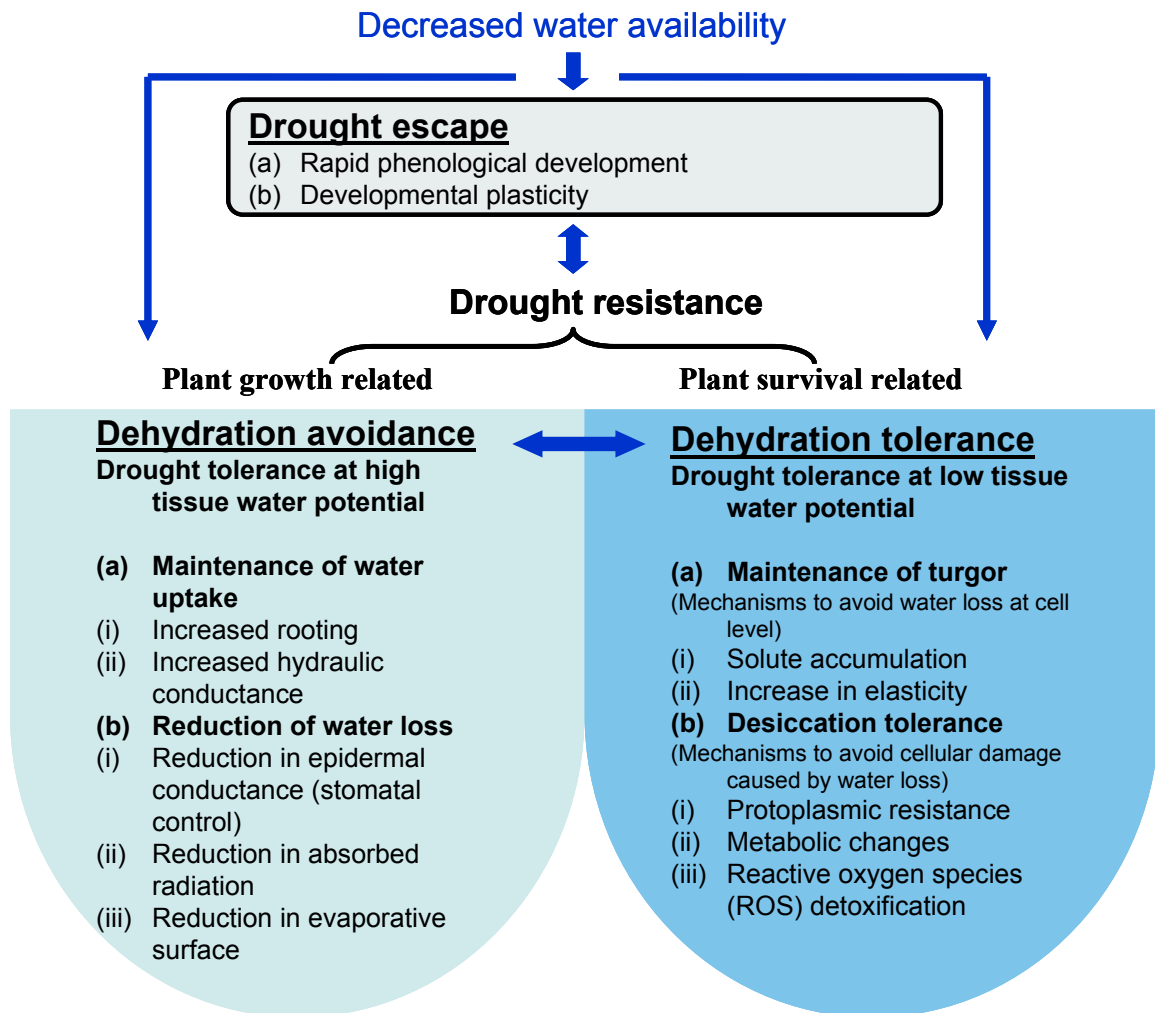


Fig. 2. 1: Conceptual diagram of crop plant adaptation mechanisms in response to decreased water availability. Information in this diagram adapted from May and Milthorpe (1962), Levitt (1972), Jones *et al.*, (1981) and Verslues *et al.* (2006).

while earlier maturing genotypes were better adapted under stress conditions. This is generally true for terminal or late season drought stress.

In contrast, there are also several studies which confirmed the positive association of long growth duration and yield potential. It is, therefore, believed that some of the yield potential is sacrificed in return for early phenological development under stress. This could be a serious problem in environments where the moisture pattern is unpredictable. This could also be a drawback, especially in indeterminate plants that offer a potential for re- growth and productivity upon recovery (Bidinger *et al.*, 1982; Turk *et al.*, 1980; Villalobos-Rodruiguez and Shibles, 1985). The more predictable the environment, the more crop duration can be optimized. Reduced yield potential in early maturing genotypes may be compensated to some extent by increasing plant density (Blum, 1970).

2.1.2 Dehydration avoidance

Dehydration avoidance (as termed by Levitt, 1972) or drought tolerance at high tissue water potential, can be simply defined as the plant's ability to retain a relatively higher level of water potential under soil and atmospheric water stress. In most cases, a plant's first response to water stress is to avoid low tissue water potential. This is achieved by increasing water uptake or limiting water loss in order to maintain the water balance. Perhaps the first response of a plant to stress is limiting water loss mainly by stomatal closure. In the longer term, changes in root and shoot growth are of greatest importance for crop plants. According to Kramer and Boyer (1995), avoidance mechanisms can be sufficient to maintain plant performance.

Reduction of water loss through stomatal control is linked with reduction in carbon gain by the plant; hence a reduction in water loss through stomatal control also results in a reduction in assimilation with consequent effects on productivity. Other mechanisms for the control of water loss include the reduction in radiation load via change in plant canopy architecture (Mooney *et al.*, 1977) and reduction in evaporative surface area (McMichael *et al.*, 1973; Constable and Hearn, 1978).

2.1.3 Dehydration tolerance

When water stress becomes more severe and the plant tissue is not protected from dehydration by avoidance mechanisms, cells lose turgor and dehydrate. Cellular dehydration causes significant cellular structural alterations (Poljakoff-Mayber, 1981). Mechanisms related to dehydration tolerance are more or less related to survival mechanisms and not productivity. The ability of tissue to maintain turgor pressure during severe water stress is an important mechanism of dehydration tolerance (Hsiao, 1973; Hsiao *et al.*, 1976).

Most of the dehydration tolerance traits studied are primarily involved with protection of cellular structure from the effect of dehydration. Several types of protective proteins including dehydrins and late-embryogenesis abundant (LEA) proteins are known to be accumulated in response to decrease in tissue water content (Close, 1997). These proteins act as chaperones that protect protein and membrane structure (Bravo *et al.*, 2003; Hara *et al.*, 2001). Compatible solutes can also protect protein and membrane structure under dehydration (Hinch and Hagemann, 2004). The role of reactive oxygen

species (ROS) in stress signaling have been extensively studied in recent years and reviewed (Chen and Gallie, 2004; Hung *et al.*, 2005).

An important point for discussion at this stage is that different drought mechanisms do not necessarily occur in a linear progression in time after the stress begins or from mild stress to severe stress. For example, some decrease in water content and turgor is required to trigger accumulation of abscisic acid (ABA) (Pierce and Raschke, 1980; Creelman and Zeevaart, 1985) which then causes stomatal closure to prevent further decrease in water content. Another important point to consider is that stressful environments are often characterized by the simultaneous or sequential occurrence of more than one stress. For example, salinity is often associated with drought or water logging, and drought is often associated with high temperature. Perennial crops in some areas may experience summer droughts and winter cold. The tolerance of plants to stress has been widely shown to vary with physiological growth stage, developmental stage and size of the plants. There is also growing evidence of multiple tolerances to stresses in plants, with plants showing tolerance to more than one stress. There are also evidences of cross-adaptation, where tolerance from one stress enhances the tolerance against other stress. For example, ABA increases tolerance against cold/drought and also enhances tolerance against diseases through increasing the thickness of the cell wall.

The consideration of avoidance versus tolerance mechanisms depends upon the objectives of the researcher and the pattern of drought stress or host organism. Plant breeders and agronomists may be interested in drought resistance mechanisms related to productivity (drought escape and dehydration avoidance) while ecologists may be interested in mechanisms related to survival (dehydration tolerance).

2.2 Drought and chickpea

Drought is the most common abiotic stress limiting chickpea production in different parts of the world. Chickpea frequently suffers from drought stress towards the end of the growing season in rain-fed conditions. Ninety percent of the world's chickpea is produced in areas relying upon conserved, receding soil moisture. Therefore, crop productivity is largely dependent on efficient utilization of available soil moisture (Kumar and Van Rheenen, 2000). Although chickpea is known for its better drought tolerance than most other cool-season legumes, drought does reduce yields and can even lead to total crop failure. In both Mediterranean and sub-tropical climates, seed filling in chickpea is subject to terminal drought, which limits seed yield (Turner *et al.*, 2001).

In chickpea, the focus of drought resistance research is on the ability to sustain greater biomass production and crop yield under seasonally increasing water deficit, rather than the physiological aptitude for plant survival under extreme drought shock (Serraj and Sinclair, 2002). This has led to the focus on escape and avoidance strategies such as early maturity (Kumar and Abbo, 2001) and large root systems (Saxena *et al.*, 1995; Singh *et al.*, 1995; Kashiwagi *et al.*, 2006).

Research into the plant response to water stress is becoming increasingly important, as most climate change scenarios suggest an increase in aridity in many areas of the globe (Petit *et al.*, 1999). On a global basis, drought, in conjunction with coincident high temperature and radiation, poses the most important environmental constraint to plant survival and crop productivity (Boyer, 1982). Agriculture is a major user of water resources in many regions of the world. With increasing aridity and a growing population, water will become an even scarcer commodity in the near future, thus a better

understanding of the effects of drought on plants is vital for improved management practices and breeding efforts in agriculture.

2.3 Physiological approaches for yield improvement

An important question in plant biology is the role of physiological traits in plant adaptation and performance under diverse environmental conditions. Water limitation is one of the important factors limiting crop productivity worldwide. Nearly all terrestrial plants are exposed to drought stress at different times and to different intensities during their life cycle (Stebbins, 1952; Bohnert *et al.*, 1995; Bray, 1997). As water is fundamental to almost all aspects of plant growth, plants are thought to have evolved numerous strategies for coping with limited water availability including changes in phenological developmental and physiological traits (Schulze *et al.*, 1987; Ludlow, 1989; Ehleringer and Monson, 1993; Ingram and Bartels, 1996; Passioura, 1996; Geber and Dawson, 1997; Ackerly *et al.*, 2000; Araus *et al.*, 2002).

The first plant stress symptom induced by drought is often rapid inhibition of shoot and root growth. This is closely followed by partial or complete stomatal closure, with reductions in transpiration and CO₂ uptake for photosynthesis. If not relieved, drought then leads to interrupted reproductive development, premature leaf senescence, wilting, desiccation and death (Hsaio, 1973; Schulze, 1986).

Breeding efforts for improvement of drought tolerance in crop plants is primarily based on selection for grain yield under drought stress. Because of the variability in drought pattern from year to year, further progress may not be achieved by selecting solely for grain yield. Although the influence of drought on chickpea yield has been

documented, research on the physiological responses of chickpea to water stress is limited (Sheldrake and Saxena, 1979). To overcome the low response to a direct selection for yield under drought conditions, substantial efforts have targeted the manipulation of morpho-physiological traits influencing drought resistance through escape, avoidance and / or tolerance mechanisms (Ludlow and Muchow 1990; Blum 1996).

2.3.1 Early maturity and root system in chickpea

Early maturing chickpea varieties that escape terminal drought have been developed (Kumar and Abbo, 2001), but early maturity places a ceiling on the potential yield and limits the crop's ability to exploit extended growing periods. Increasing the drought avoidance of the crop should help to stabilize yields at higher levels than possible with escape (Johansen *et al.*, 1997).

Effects of deeper rooting systems on sorghum yield have been confirmed by simulation studies across a number of years and environments in USA (Sinclair, 1994). Similarly, a simulation model has been adapted for chickpea and used to predict crop yield potential (Soltani *et al.*, 1999). In this model, increase in crop biomass was calculated from the quantity of solar radiation intercepted by the leaf canopy multiplied by crop radiation-use efficiency (RUE). A soil water budget was included in the model to account for the potential inhibition of water availability on phenological development, leaf growth and senescence, and biomass accumulation under water limited conditions. This model showed that early maturity and increasing drought avoidance via deep roots, plus higher transpiration efficiency were the traits most likely to result in higher grain yield under terminal drought stress (Soltani *et al.*, 2000).

Roots have a major role in dehydration avoidance as deep root system is able to obtain moisture from the deeper soil layers even when the upper soil layer becomes dry. Sponchiado *et al.*, (1980) and Pandey *et al.*, (1984) hypothesized that the ability of a plant to change its root distribution in the soil is an important mechanism for drought avoidance. Pandey *et al.* (1984) reported that peanut and cowpea were able to change root distribution in the soil because of dry conditions and extracted water from greater depths than soybean and mung bean. Benjamin and Nielsen (2006) reported that greater root surface area to weight ratio in chickpea as compared to field pea and soybean indicates either a finer root system or roots with lower specific density. Sponchiado *et al.* (1980) reported that the ability of common bean to change root distribution to avoid drought stress varied by cultivar. One can also think about efficient root system in comparison with large root system as it also has offset by a fall in harvest index because there is much less assimilate available for grain growth.

Studies in various crops have shown the importance of a deep root system for extracting moisture under terminal drought stress (Ludlow and Muchow, 1990; Saxena and Johansen, 1990; Turner *et al.*, 2001). Kashiwagi *et al.* (2006) found substantial variation in root length density among 12 diverse kabuli and desi chickpea genotypes at different soil moisture levels. The proportion of the roots at the lower depth was also important in water absorption from deeper soil layers. They also found close association of genotypic performance under 70% field capacity cylinder in greenhouse with that of the field conditions suggests that the cylinder protocol could be adapted for screening studies of root traits. Roots at the deeper soil layer contributed more to root length or surface area than to root weight (Follett *et al.*, 1974). Deep root systems in sorghum

demonstrated increased yield under drought conditions (Jordan *et al.*, 1983; Sinclair, 1994). In rice, deep root morphology was associated with increased water extraction during progressive water stress (Fukai and Cooper, 1995; Kamoshita *et al.*, 2002). A high ratio of deep root weight to shoot weight also maintained higher plant water potential and had a positive effect on yield under drought stress conditions (Mambani and Lal, 1983). Current research on rice is focused on the use of molecular markers for various root traits including rooting depth, root volume, root thickness to improve drought avoidance (Cui *et al.*, 2002; Price, 2002).

Field studies in legumes (Saxena and Johansen, 1990; Turner *et al.*, 2001) showed that both dense root systems extracting more of the water in upper soil layers and longer root systems extracting soil moisture from deeper soil layers are important for maintaining yield under terminal drought stress. A higher ratio of deep root weight to shoot weight was also found to maintain higher plant water potentials and have a positive effect on yield under stress (Mambani and Lal, 1983). Ludlow and Muchow (1990) recommended traits that are suited for intermittent stress conditions in modern agriculture. Their top three recommendations in order of priority were to match plant phenology to water supply, osmotic adjustment, and rooting depth.

2.3.2 Stomatal conductance

2.3.2.1 Stomatal Movement

Stomata are openings through which gases diffuse into and out of leaves (Fig. 2.2). Stomata also provide a means of controlling water loss from plants while allowing photosynthesis. Consequently, stomata have a major role in the biological control of our

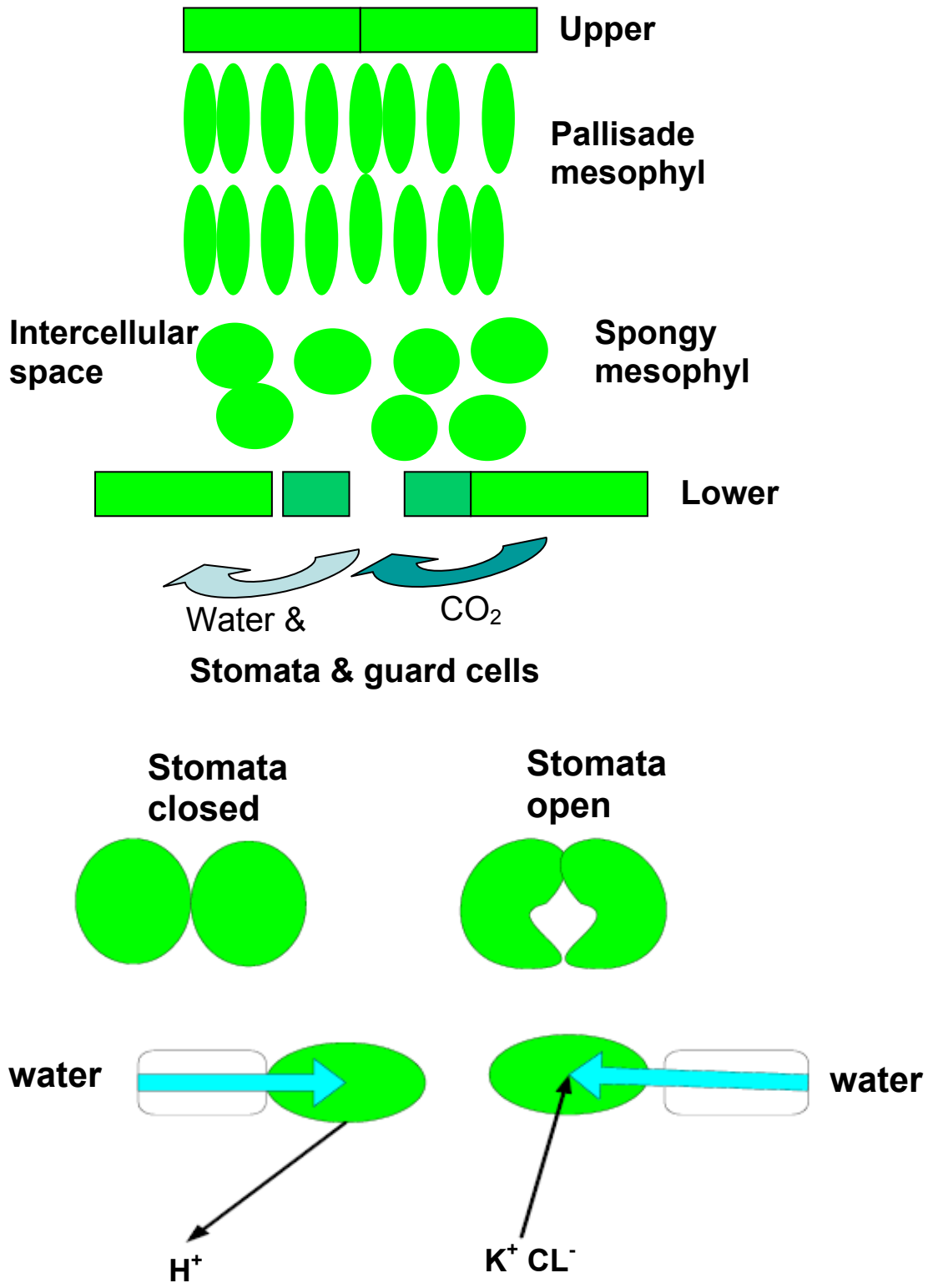


Fig. 2. 2: Ion exchange and stomatal mechanics. Adapted from Nobel P.S. (2005)

climate system and the chemistry of our atmosphere. The aperture of the stomatal pore must be finely tuned in order to allow uptake of sufficient CO₂ yet not to lose excessive water to desiccate plants. This fine-tuning process is controlled by a pair of guard cells that surround each stomatal pore. When guard cells swell due to increased turgor pressure, the pore aperture enlarges. When guard cells lose turgor pressure and shrink, stomatal pores become smaller. The turgor pressure of guard cells is regulated by solute concentration and water flow across cell membranes. Major solutes in guard cells include K⁺, Cl⁻, and malate. Luan (2002) summarized signaling in guard cells in relation to drought.

The transpiration path involves evaporation within the leaf at the walls of the palisade and spongy parenchyma cells from where it diffuses into the intercellular spaces, the substomatal cavity and then out of the stomata. A reversed path occurs for carbon dioxide (CO₂). Stomatal movement is primarily a result of turgor changes in the guard cells. This change in turgor occurs due to uptake of water by guard cells. Many processes are linked with stomatal opening. Turgor adjustment in adjacent epidermal cells due to inorganic ions can also force stomatal movement. The movement of inorganic ions is not the only factor causing turgor changes, malate can also participate. In addition to the movement of anions and cations, ABA also acts to open and close stomata. During a drought period, stomatal control involves a metabolic signal from the roots (Gollan *et al.*, 1986; Schulze *et al.*, 1987).

The opening and closing of stomata involve feedback and feed forward loops (Jones, 1998).

1. A decrease of CO_2 in the intercellular air space and guard cells enables K^+ to move into the guard cells and open the stomata. This allows CO_2 to diffuse into the leaf for photosynthesis. Conversely, stomata close when exposed to elevated CO_2 levels.
2. If transpiration rates are high (e.g. due to low humidity or a high radiation load) a direct feed forward effect occurs by altering the turgor of the guard cells, causing stomata to close.
3. When soils dry, an increase in ABA is noted in the transpiration stream. This forces a closing of stomata, to conserve water.

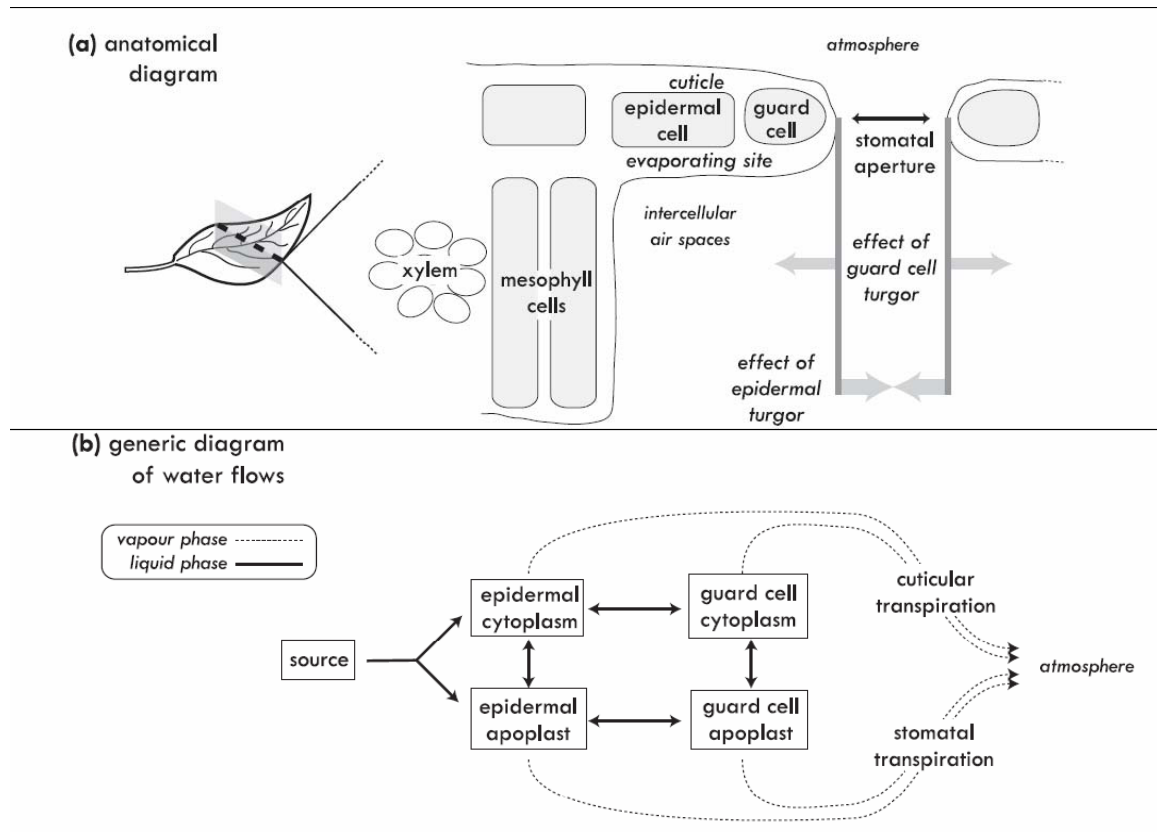


Fig. 2. 3: Diagram showing (a) anatomical situation of structures indicating the opposing effects of guard and epidermal cell turgor pressure on stomatal aperture, and (b) water-exchange compartments associated with the stomatal complex, and possible flow among them. (Buckley, 2005).

2.3.2.2 Stomatal conductance measurement

The control that stomata exert on water vapour transpiration, carbon assimilation and respiration is expressed in terms of the stomatal conductance or its inverse, the resistance. It is a property that relates the conductance across a unit area of leaf, so it does not correspond to the efforts of single stomata.

Stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) was measured with a steady state porometer (Li-1600, LI-COR Inc., Lincoln, NE) in this study. The Li-Cor 1600 operates on the null balance principal. When an intact transpiring leaf is inserted in the cuvette (Fig. 2.4), it raises humidity inside the cuvette. To balance this increased humidity, the internal flow controller increases the flow of dry air to the cuvette until it balances the humidity to a predetermined set point which is ambient relative humidity. As different genotypes transpire at different rates, different amounts of dry air are required to reach the set point. This difference is used to compute the stomatal conductance.

For most plants, drought avoidance is achieved primarily through regulation of stomatal conductance in response to soil and atmospheric water deficit (Cohen, 1970; Cowan, 1982; Schulz, 1986; Dawson and Ehleringer, 1993; Meinzer, 1993). Stomatal closure can serve as a rapid and effective drought avoidance response. However, prolonged stomatal closure is not sustainable as stomatal CO_2 uptake is also reduced and will ultimately limit photosynthetic assimilation and growth (Farquhar and Sharkey, 1982; Schulze *et al.*, 1987).

Ritchie *et al.* (1990) observed in wheat that the most drought resistant genotypes had greater stomatal conductance under water-stress conditions than the susceptible genotypes. Yield was improved under drought by higher stomatal conductance in wheat

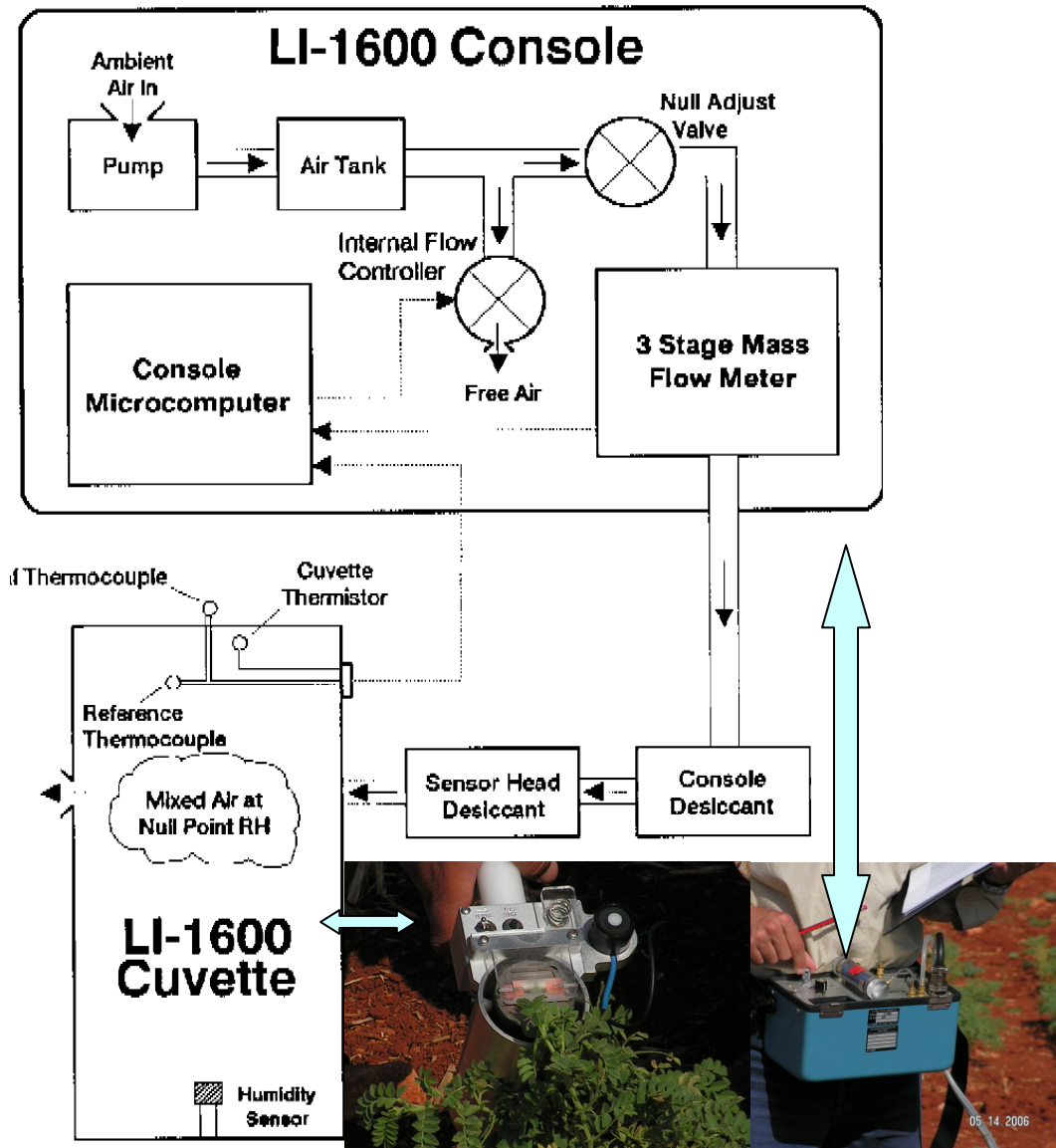


Fig. 2. 4: A schematic diagram of the operation of LI-1600 porometer to measure stomatal conductance (Li-1600 operations manual, LI-COR Inc., Lincoln, NE)

(Fischer *et al.*, 1998). González *et al.*, (1999) also found a strong association between barley yield under drought and higher stomatal conductance. Hence, selection of genotypes for higher stomatal conductance under drought stress could help to improve yield under drought stress.

2.3.3 Canopy temperature

An important consequence of the stomatal closure that occurs when plants are subject to water stress is that energy dissipation is decreased so leaf temperature tends to rise. The idea of using leaf or canopy temperature as an indicator of plant water stress is not a new one (e.g. Tanner, 1963; Idso *et al.*, 1981; Jackson *et al.*, 1981). Since a major role of transpiration is leaf cooling, canopy temperature and its reduction relative to ambient temperature is an indication of the role of transpiration in cooling the leaves. The relationship among canopy temperature, air temperature and transpiration is considered when canopy temperature is used to develop the crop water stress index (CWSI), which is gaining importance in irrigation scheduling in crops.

However, interest is also increasing in using canopy temperature in plant breeding for drought tolerance. The goal is to select genotypes that maintain lower canopy temperature as compared with other genotypes under the same field conditions. Relatively lower canopy temperature in drought stressed crop plants indicates a relatively better capacity for taking up soil moisture and for maintaining a relatively better plant water status. Canopy temperature was considered to be effective in screening wheat (Blum *et al.*, 1982; Pinter *et al.*, 1990) and pearl millet (Singh and Kanemasu, 1983) genotypes for resistance to drought. Chaudhuri and Kanemasu (1982) found that yields of

sorghum hybrids were negatively correlated with the seasonal average canopy temperature and canopy – air temperature differences. Similar results have also been reported for potato (Stark and Pavek, 1987).

Canopy temperature is generally measured remotely by infrared thermometry (IRT). Plant canopies emit infrared radiation as a function of temperature. The infrared thermometer senses this radiation and converts it to an electrical signal which is displayed as temperature. A hand-held infrared thermometer model 100.3ZL (Everest Interscience Inc., Fullerton, CA) with 4° field of view, was used in this study to measure canopy temperature.

2.3.4 Chlorophyll fluorescence analysis

2.3.4.1 The basics of chlorophyll fluorescence

Photosynthesis is an essential process to maintain crop growth and development. Photosynthetic organisms use light energy to produce organic molecules (Ort and Whitmarsh, 2001). The photosynthetic process (Fig. 2.5) depends on photosystem II (PSII), a membrane-bound protein complex that removes electrons from water and transfers them to plastoquinone (PQ). PSII is the only protein complex known to oxidize water and release molecular oxygen. PSII is linked with photosystem I (PSI) by the cytochrome *bf* complex and small mobile electron carriers (Whitmarsh and Govindjee, 1999). PSII, the cytochrome *bf* complex and PSI are embedded in the thylakoid membrane and operate in series to transfer electrons from water to NADP⁺. The energy required to move electrons is provided by light, which is captured by light harvesting antenna complexes of PSII and PSI.

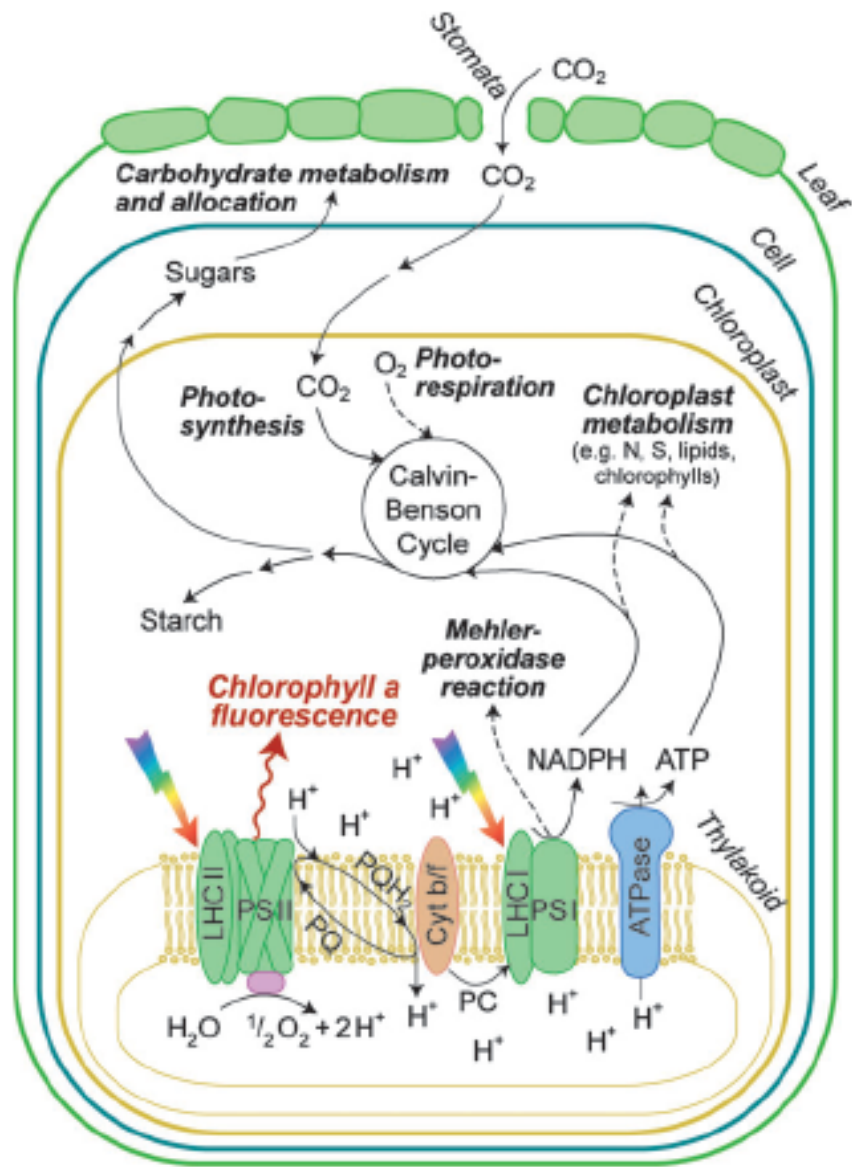


Fig. 2. 5: Schematic overview of photosynthesis showing main processes in C3 plants. (Baker and Rosenqvist, 2004)

The principle of chlorophyll fluorescence analysis is quite simple. Light energy absorbed by chlorophyll molecules can undergo one of three fates (Fig.2.6).

- (a) PSII uses light energy to drive chemical reactions (photochemistry), e.g., oxidation of water and the reduction of plastoquinone (Govindjee and Coleman, 1990; Nugent, 2001).
- (b) Excess energy can be dissipated as heat or
- (c) It can be re-emitted as light, which is termed as chlorophyll fluorescence.

These three processes occur in competition with each other. Increase in the efficiency of one will result in the decrease of other two. By measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be obtained.

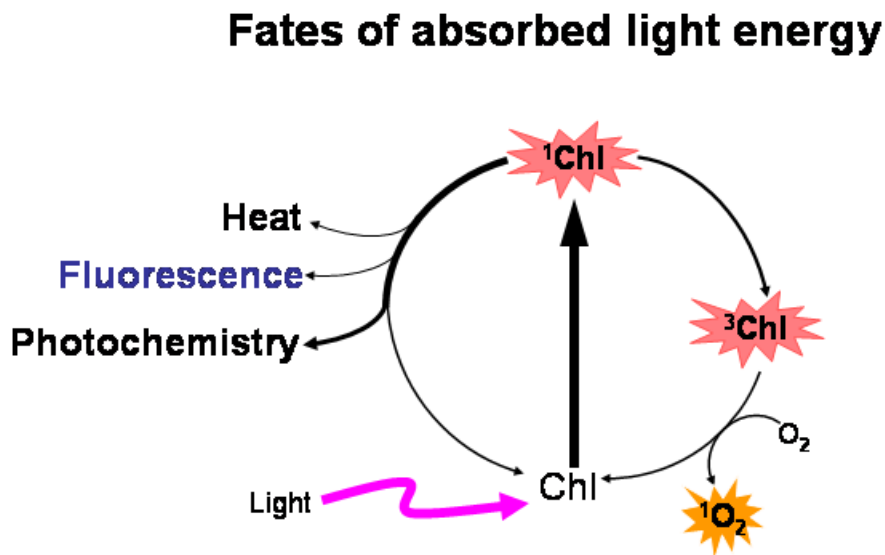


Fig. 2. 6: Schematic drawing showing fate of light energy absorbed by chlorophyll molecules in a leaf. (Drawing from Khanal, N., Plant Sciences, University of Saskatchewan, Canada, personal communication)

2.3.4.2 Chlorophyll fluorescence parameters

There are many fluorescence parameters defined in the literature. The parameters that were used in this study are reviewed here. For the measurement of chlorophyll fluorescence, it is important to ‘switch off’ the process of photochemistry in order to measure fluorescence yield. For this purpose, a method has been developed called ‘light doubling’ that allows the contribution of photochemical quenching to be transiently reduced to zero (Bradbury and Baker, 1981; Quick and Horton, 1984). During the induction of photosynthesis when a dark-adapted leaf is exposed to light, large changes in chlorophyll fluorescence occur. On immediate exposure to light (Fig.2.7), fluorescence rises to the minimal level, termed F_o level, which is the fluorescence level obtained when the PSII reaction centers are in the ‘open’ state (capable of photochemistry since QA, the

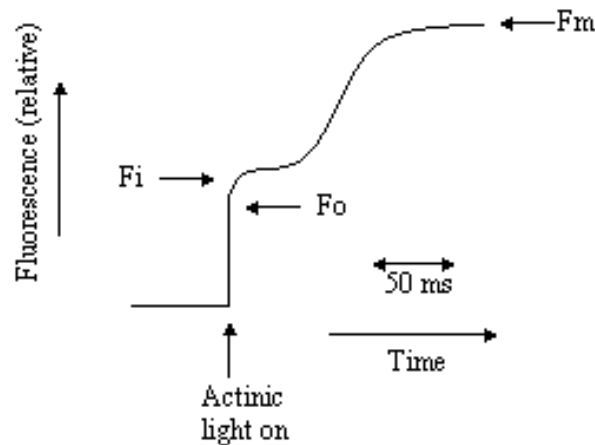


Fig. 2. 7: Simple scheme of chlorophyll fluorescence induction in a dark-adapted leaf exposed to weak actinic blue light ($30 \mu\text{mol m}^{-2}\text{s}^{-1}$). The minimal (F_o), transient inflection (F_i), and maximum (F_m) levels of fluorescence are shown. (Adapted on the basis of information from Maxwell and Johnson, 2000; Baker and Rosenqvist, 2004)

primary quinone acceptor of PSII, is maximally oxidized). The fluorescence then raises rapidly to the transient inflection level or the steady-state yield of fluorescence in the light (F_i), before reaching a peak level i.e. the maximum fluorescence (F_m , in the absence of photochemical quenching). When fluorescence reaches the F_m level, the reaction centers are said to be closed and the plastoquinone pool is fully reduced. The difference between F_m and F_o is termed as variable fluorescence (F_v) and the ratio F_v/F_m depicts the maximum quantum efficiency of PSII.

Photosystem II (PSII) is an important component of plant photosynthesis, and is particularly sensitive to water deficit (Lu and Zhang, 1999). Chlorophyll fluorescence is widely accepted as an indication of the energetic behavior of a photosynthetic system. With the decrease in the relative water content of leaves, stomata close, imposing a decrease in the supply of CO_2 to the mesophyll cells and ultimately decreases the rate of leaf photosynthesis (Williams *et al.*, 1999; Lawlor and Cornic, 2002; Araus *et al.*, 1998; Fracheboud *et al.*, 2004). Parameters, such as F_o and F_m measured during the grain filling stage of wheat under drought stress showed high genetic correlation with grain yield (Araus *et al.*, 1998), suggesting that these parameters can be used as indicators to evaluate the yield performance across genotypes under water deficit conditions (Araus and Hogan, 1994). Hence, these parameters may be considered as traits associated with drought tolerance. However, little is known about the possibility of screening large populations under field drought conditions using these parameters. A better understanding of the genetic basis of these parameters and their association with drought tolerance will contribute to their possible use in breeding strategies for dry environments.

2.4 Chickpea genome mapping

2.4.1 DNA marker systems for chickpea

In recent years, the use of molecular markers has facilitated breeding of crop plants (reviewed by Melchinger, 1990; Winter and Kahl, 1995; Charcosset and Moreau, 2004; Millan *et al.*, 2006; Coram *et al.*, 2007), including breeding for biotic and abiotic stresses. Molecular marker technology has made it possible to generate genetic maps of chickpea that holds promise for use in marker-assisted selection and positional cloning of agronomically important genes. Cultivated chickpea has limited genetic polymorphism (Ahmad and Slinkard, 1992; Udupa *et al.*, 1993; Labdi *et al.*, 1996). The availability of sufficient polymorphic markers is a prerequisite for successful linkage studies. Commonly used markers such as isozymes (Kazan and Muehlbauer, 1991), restriction fragment length polymorphisms (RFLP) (Udupa *et al.*, 1993; Simon and Muehlbauer, 1997), and random amplified polymorphic DNA (RAPD) (Simon and Muehlbauer, 1997) generally failed to reveal intraspecific variations in chickpea and their use for marker assisted selection (MAS) is therefore limited. Markers that are polymorphic within cultivated chickpea are needed for MAS.

Microsatellites (Tautz and Rentz, 1984), also known as simple sequence repeat (SSR) markers, are DNA-based molecular markers that offer several advantages because they are reproducible, polymorphic, co-dominant, polymerase chain reaction (PCR)-based and readily portable within a species (Edwards *et al.*, 1996; Dib *et al.*, 1996; Powel *et al.*, 1996) and are amenable to automated, non-radioactive detection (Mansfield *et al.*, 1994). The variability of microsatellites is exploited by a PCR-based technique that uses microsatellite-flanking sequences as primers to amplify the microsatellites in between.

The resulting locus-specific amplification products often exhibit considerable length differences due to variations in the number of tandem repeats within the microsatellite (Litt and Luty, 1989). After these so-called sequence tagged microsatellite site (STMS) markers (Bechmann and Soller, 1990) had been successfully employed for the generation of a high – density marker map of the human genome (Weissenbach *et al.*, 1992), they were also widely applied to plant genome analysis. SSR markers have been generated for many major crops species (see Powel *et al.*, 1996 for review), including bean, pea and soybean (Akkaya *et al.*, 1992; Akkaya *et al.*, 1995; Maughan *et al.*, 1995; Rongwen *et al.*, 1995). Recent studies reported the cloning and characterization of polymorphic microsatellite sequences from *Cicer arietinum* (Hüttel *et al.*, 1999; Winter *et al.*, 1999).

2.4.2 Chickpea genetic mapping

Genetic linkage maps of chickpea (*Cicer arietinum* L.) have been published using morphological isozymes (Gaur and Slinkard, 1990; Kazan *et al.*, 1993), RFLP and RAPD (Simon and Muehlbauer, 1997), STMS, amplified fragment length polymorphism (AFLP) (Winter *et al.*, 1999), morphological isozyme, inter simple sequence repeat (ISSR) and RAPD loci (Santra *et al.*, 2000), STMS markers (Tekeoglu *et al.*, 2002; Flandez-Galvez *et al.*, 2003; Udupa and Baum, 2003; Cho *et al.*, 2004; Tar'an *et al.*, 2007). Because of the common markers in the last five chickpea maps, map integration from different studies is possible. Tekeoglu *et al.* (2002) developed a chickpea map from 65 STMS primer pairs and a population size of 142 RILs from a interspecific cross between FLIP84-92C (*Cicer arietinum*) and PI599072 (*Cicer reticulatum*). They also integrated this map with marker data from Santra *et al.* (2000) and reported a total genetic

map with 167 markers on nine linkage groups covering 1,174.5 cM with an average distance of 7.0 cM between markers. The genetic map reported by Flandez-Galvez *et al.*, (2003) was based on 66 markers including 51 SSRs and a population of 85 F₂ plants from an intraspecific cross between desi cultivars ICC12004 and Lasseter. Udupa and Baum (2003) generated a map from 52 SSRs and a population size of 97 RILs from an intraspecific cross kabuli type chickpea between ILC 1272 and ILC 3279. The genetic map reported by Cho *et al.*, (2004) was generated from 53 STMS primer pairs based on the population of RILs from a cross between PI359075(1) and FLIP84-92C(2). The recent genetic map published by Tar'an *et al.* (2007) was generated from 135 primer pairs including 134 SSRs and was based on a population of 186 F₂ plants from an intraspecific cross of desi cultivar ICCV96029 and kabuli cultivar CDC Frontier. Markers reported in this map were assigned to 8 linkage groups with a combined linkage distance of 1,285 cM. The average linkage distance between primer pairs in all linkage groups was 8.9 cM. Common markers in these and future maps with SSR primer pairs could lead to the development of a high density genetic map of chickpea to identify tightly linked flanking markers for genes of interest, which ultimately will be helpful in marker-assisted selection (MAS) and positional cloning of agronomically important genes.

2.5 Identification of QTLs related to chickpea drought tolerance

Compared to the conventional breeding approaches for improved productivity under water limited environments, genomics offers great opportunities for dissecting quantitative traits into their single genetic determinants (Young, 1996; Dudley, 1993; Tanksley, 1993; Lee, 1995; Beavis and Kein, 1996; Quarrie, 1996; Prioul *et al.*, 1997;

Tuberosa *et al.*, 2002). Identification of QTLs is paving the way to MAS (Ribaut *et al.*, 2002; Morgante and Salamini, 2003) and assisted pyramiding of the beneficial QTL alleles. Marker-assisted breeding reduces the effect of environmental conditions during the selection process, which is a major hindrance in conventional breeding under drought. The increasing number of studies reporting QTLs for drought related traits in different crops under drought stress (Table-2.1) indicates a growing interest in this approach. With the invention of other genomic tools, sequencing and bioinformatics, new dimensions for deciphering and manipulating the genetic basis of drought tolerance can be achieved (Tuberosa *et al.*, 2002; Varshney *et al.*, 2005; Tuberosa *et al.*, 2005).

Although considerable progress has been made in identifying QTLs in chickpea related to fusarium wilt and ascochyta blight disease resistance (Table 2.2), information on the genetic basis of traits related to drought tolerance in chickpea is limited. The International Crops Research Institute for the Semi-arid Tropics (ICRISAT) is deploying MAS to introgress QTL alleles associated with large root size into elite germplasm of chickpea (Saxena *et al.*, 2000). A deep root system capable of extracting additional soil moisture should positively impact yield under drought stress environments. A set of 257 RILs was developed from the cross of Annigeri x KC4958 at ICRISAT and evaluated to identify molecular markers for root traits. Over 250 STMS and 100 EST markers were initially screened on parents of the RILs. Fifty seven STMS markers detected polymorphism and were mapped on the RILs population. A QTL flanked by marker

Table 2. 1: Summary of some recent QTL studies related to drought in different crops.

Crop	Cross	Environment	Main trait (s)	Reference
Arabidopsis	Landsberg _ Cape Verde	Greenhouse	Stomatal conductance, transpiration efficiency, flowering time	Juenger <i>et al.</i> , 2005
Barley	Tadmor _ Er/Apm	Field	Osmotic adjustment, leaf relative water content, grain yield	Diab <i>et al.</i> , 2004
Cotton	G. hirsutum _ G. barbadense	Field	Canopy temperature, osmotic potential, dry matter, seed yield	Saranga <i>et al.</i> , 2004
Maize	F2 _ F252	Field	Silking date, grain yield, yield stability	Moreau <i>et al.</i> , 2004
Maize	Os420_IABO78	Field	Stomatal conductance, drought sensitivity index, leaf temperature, leaf relative water content, anthesis-silking interval, grain yield	Sanguineti <i>et al.</i> , 1999
Rice	Zhenshan97B_Milyang 46	Field	Chlorophyll content	Shen <i>et al.</i> , 2007
Rice	CT9993. _ IR62266	Field	Root morphology, plant height, grain yield	Chandra Babu <i>et al.</i> , 2003
Rice	IR62266. _ IR60080.	Greenhouse	Osmotic adjustment	Robin <i>et al.</i> , 2003
Rice	IRAT109 _ Yuefu	Field / pots	Root traits	Li <i>et al.</i> , 2005
Rice	Teqing _ Lemont	Field	Phenology, yield components	Xu <i>et al.</i> , 2005
Rice	Nipponbare_ Kasalath	Field	Stomatal frequency, leaf rolling	Ishimaru <i>et al.</i> , 2001
Rice	Azucena _ Bala.	Pots	Stomatal conductance, leaf rolling, heading date	Price <i>et al.</i> , 1997
Sorghum (grain)	B35 _ Tx7000	Field	Stay green, chlorophyll content	Xu <i>et al.</i> , 2000
Wheat	SQ1 _ Chinese spring	Field	Water use efficiency, grain yield	Quarrie <i>et al.</i> , 2005
Wheat	Beaver _ Soissons	Field	Flag leaf senescence	Verma <i>et al.</i> , 2004

Table 2. 2: Summary of QTL studies related to diseases in chickpea.

Cross	Type	Environment	Main trait(s)	Reference
ICCV96029_CDC Frontier	Desi_Kabuli	Greenhouse	Ascochyta blight	Tar'an <i>et al.</i> , 2007
ILC72_Cr5-10	<i>C. arietinum</i> _ <i>C. reticulatum</i>	Field	Ascochyta blight	Cobos <i>et al.</i> , 2006
ILC3279_WR315	Kabuli_desi	Field	Ascochyta blight	Iruela <i>et al.</i> , 2006
Hadas _ ICC 5810	Kabuli_desi	Field	Ascochyta blight, time of flowering	Lichtenzveig <i>et al.</i> , 2006
PI359075_FLIP84-92C	<i>C. arietinum</i>	Greenhouse	Ascochyta blight	Cho <i>et al.</i> , 2004
FLIP84-29C _ PI599072	<i>C. arietinum</i> _ <i>C. reticulatum</i>	Field	Ascochyta blight	Tekeoglu <i>et al.</i> , 2002
Lasseter_PI527930	<i>C. arietinum</i> _ <i>C.echinospermum</i>	Greenhouse	Ascochyta blight	Collard <i>et al.</i> , 2003
ICC12004 _ Lasseter	Desi	Field, Greenhouse	Ascochyta blight	Flandez-Galvez <i>et al.</i> , 2003
ILC3279_CA2156	<i>C. arietinum</i>	Field and Greenhouse	Ascochyta blight	Millan <i>et al.</i> , 2003
ICC4958_PI489777	<i>C. arietinum</i> _ <i>C. reticulatum</i>	Greenhouse	Ascochyta blight	Rakshit <i>et al.</i> , 2003
FLIP84-92C_PI599072	<i>C. arietinum</i> _ <i>C. reticulatum</i>			
ILC1272_ILC3279	Kabuli	Growth chambers	Ascochyta blight	Udupa and Baum, 2003
FLIP84-92C_PI599072	<i>C. arietinum</i> _ <i>C. reticulatum</i>	Field	Ascochyta blight	Santra <i>et al.</i> , 2000
PI359075_FLIP84-92C(2)	<i>C. arietinum</i>	Field	Ascochyta blight	Tekeoglu <i>et al.</i> 2000a
Blanco Lechoso_Dwelley	<i>C. arietinum</i>			
FLIP84-92C_PI599072(3)	<i>C. arietinum</i> _ <i>C. reticulatum</i>			
CA2156_JG62	Kabuli_desi	Field	Fusarium wilt	Cobos <i>et al.</i> , 2005
CA2139_JG62	Kabuli_desi			
C104_WR315	<i>C. arietinum</i>	Greenhouse	Fusarium wilt	Sharma <i>et al.</i> , 2004
ICC4958_PI489777	<i>C. arietinum</i> _ <i>C. reticulatum</i>	Greenhouse	Fusarium wilt	Tekeoglu <i>et al.</i> 2000b
ICC-4958 _ PI498777	<i>C. arietinum</i> _ <i>C. reticulatum</i>	Greenhouse	Fusarium wilt	Winter <i>et al.</i> , 2000
C-104_WR-315	<i>C. arietinum</i>	Greenhouse	Fusarium wilt	Tullu <i>et al.</i> , 1998
C104_WR315	<i>C. arietinum</i>	Greenhouse	Fusarium wilt	Mayer <i>et al.</i> , 1997

TAA170 and TR55 on LG4A identified for root length (Chandra *et al.*, 2003). A few researchers have studied the genetic basis of time of flowering in chickpea. Or *et al.* (1999) suggested a major photoperiod response gene (*Ppd*) affecting time to flowering. Cho *et al.* (2002) identified a single QTL for days to 50% flowering on LG3 with a LOD score of 3.03. Lichtenzveig *et al.* (2006) identified two QTLs on LG1 and LG2 linked to time to first flower. Cho *et al.* (2002) also identified a QTL for seed weight on LG4 accounting for 52% of the total phenotypic variation. These reports generated information on QTLs for important traits which can be used for stress breeding in chickpea. The basis of this further development could lie in the integration of physiology and biotechnology towards plant breeding (Blum and Nguyen, 2004). The characterization of key plant physiological mechanisms that restrain performance under drought, together with the associated regulatory genes, could therefore, facilitate the development by breeders of improved crop varieties showing water use efficiency and drought tolerance.

After the mapping of important QTLs, the next step is to identify candidate sequences, validate their role and proceed with the direct manipulation using the gene itself as marker for MAS (Tuberosa and Coraggio, 2004). The recent progress in the profiling of transcriptome, proteome and metablome offers the possibility of investigating the response of genes to drought and other stresses. Genetic engineering is currently being explored for enhancing the levels of drought tolerance in chickpea. ICRISAT has developed a transformation and regeneration system (Jayanand *et al.*, 2003) and transgenic plants with a dehydration responsive element (DRE) construct, where the expression of the DREB1A is driven by a drought-responsive rd29A promoter (ICRISAT, 2003). This construct is expected to enhance tolerance to several abiotic

stresses, such as drought, chilling, temperature and salinity, as it regulates a number of genes that act together in enhancing the tolerance to these stresses (Kasuga *et al.*, 1999). Plants have also been transformed with the gene PSCSF-129A that increases proline accumulation and improves tolerance to osmotic stress (Hong *et al.*, 2000). The current focus in chickpea functional genomics should be to coordinate resources around the world and take full advantage of functional genomics for crop improvement (Coram and Pang, 2007).

3. Effect of water deficit on root distribution pattern in chickpea (*Cicer arietinum* L.)

3.1 Summary

Response of the root system to water deficit was studied with a set of eight chickpea genotypes. Plants were grown in 1.2 m x 0.15 m polyvinyl chloride cylinders with three soil moisture treatments in two consecutive trials during 2005-06. Under non-stress conditions, 50% of the root biomass and root length density (RLD) was found in the 40-100 cm soil layer, while under stress conditions, 50% of the root biomass was found deeper in the 60-100 cm soil layer and RLD in the 80-100 cm layer. Genotypes ILC3279, ILC10606, ILC9955 and Amit produced significantly higher root biomass and RLD under all moisture treatments. On the other hand, higher root length to weight ratio (LWR) obtained for genotypes ILC588, ILC3182, ICCV 2 and CDC Chico indicated a finer root system or roots with lower specific density. LWR increased in the deeper layers as compared to the upper layers. A significant positive correlation was detected between root weight density and RLD with number of days to flower and maturity. Genotypes having larger RWD and RLD showed late maturity as compared to the other group, which had smaller RWD and RLD and matured early. Second group may be suitable for regions like western Canada, where crop growth termination usually required prior to fall frost.

3.2 Introduction

Chickpea (*Cicer arietinum* L.) is an important legume crop mainly grown in arid and semi-arid regions of the world (Kumar and Abbo, 2001). In most of the chickpea

growing areas, drought is a prominent characteristic which limits yield and can even lead to total crop failure. In both Mediterranean and sub-tropical climates, seed filling in chickpea is subject to terminal drought, which limits seed yield (Turner *et al.*, 2001). As water resources become limiting for crop production in dry areas, the management of drought becomes increasingly important. Drought or water deficiency can be managed at the plant level through drought escape and drought resistance mechanisms (Levitt, 1980). Drought resistance can further be described in terms of dehydration avoidance and dehydration tolerance mechanisms. Roots have a major role in dehydration avoidance as a deep root system is able to obtain more moisture from the deeper soil layers even when the upper soil layer becomes dry. Sponchiado *et al.* (1980) and Pandey *et al.* (1984) hypothesized that the ability of a plant to change its root distribution in the soil is an important mechanism for drought avoidance. Pandey *et al.* (1984) reported that peanut and cowpea are able to change root distribution in the soil because of dry conditions and extracted water from greater depths than soybean and mung bean. Benjamin and Nielsen (2006) reported that greater root surface area to weight ratio in chickpea as compared to field pea and soybean indicates either a finer root system or roots with lower specific density. Sponchiado *et al.* (1980) reported that the ability of a plant to change root distribution to avoid drought stress varied by cultivar.

Studies in various crops have shown the importance of a deep root system for extracting moisture under terminal drought stress (Ludlow and Muchow, 1990; Saxena and Johansen, 1990; Turner *et al.*, 2001). Kashiwagi *et al.* (2006) found substantial variation in root length density among 12 diverse kabuli and desi chickpea genotypes grown under terminal drought stress. The proportion of the roots at the lower depth is

also important in water absorption from deeper soil layers. Roots at the deeper soil layer contribute more to root length or surface area than to root weight (Follett *et al.*, 1974). Deep root systems in sorghum demonstrate increased yield under drought conditions (Sinclair, 1994). In rice, deep root morphology is associated with increased water extraction during progressive water stress (Kamoshita *et al.*, 2002). A high ratio of deep root weight to shoot weight also maintains higher plant water potential and has a positive effect on yield under stress conditions (Mambani and Lal, 1983). Current research on rice is focused on the use of molecular markers for various root traits like rooting depth, root volume, root thickness (diameter) to improve drought avoidance in rice (Cui *et al.*, 2002).

The study of root traits under field conditions is difficult and cumbersome and many researchers have reported the use of controlled environments to study root systems. The objective of our study was to determine the extent of genotypic differences in chickpea root systems and the effects of soil moisture stress on root distribution at various soil depths.

3.3 Materials and Methods

Eight Kabuli chickpea genotypes comprising ILC 588, ILC 3182, ICCV 2, ILC 3279, ILC 10606, ILC 9955, Amit and CDC Chico were used. These genotypes originated in different countries. The origin of ILC 588, ILC 3182, ICCV 2 is India; ILC 3279 and ILC 10606 is former USSR, ILC 9955 in Uzbekistan and CDC Chico in Canada; while Amit is cultivated in Canada but originated in Eastern Europe. Some of these genotypes were used as parents of some mapping populations being used for QTL mapping for drought and ascochyta resistance in different centers around the world

(ILC588, ILC3182, ILC3279 for drought, ILC 3279 for ascochyta blight); hence, the study of their rooting pattern could help to explain their response to other stresses.

The first trial (Trial I) was conducted during 2005 (July 13 – October 28, 2005) and was repeated (Trial II) during 2005-06 (November 23, 2005 – February 27, 2006) in the Agriculture greenhouse, University of Saskatchewan, Saskatoon, SK, Canada (52° N, 106° W). Cylinders (1.20 m length x 0.15 m diameter) were created using polyvinyl chloride (PVC) drain pipes to provide enough space for root growth for a single plant (Fig. 3.1). The base of each cylinder was closed with a perforated metal sheet to allow drainage of excess water. To facilitate root recovery, the cylinders were cut longitudinally along both sides and the joints sealed with duct tape before filling with soil.

Cylinders were filled with 1:1 mixture of potting mix (Sunshine mix # 4, Sun Gro Horticulture Canada Ltd. which contains peat moss, perlite, major and minor nutrients, gypsum, dolomitic limestone) and sand to facilitate root recovery. Three seeds were sown in each cylinder and thinned to one after emergence and seedling establishment. Seeds were treated with fungicides Carbathiin, Thiabendazole and Metalaxyl prior to sowing. Fertilizer (Plant-Prod® 20-20-20 plus micronutrients; Plant Products Company Ltd., Brampton, Ontario, Canada) was supplied to provide the equivalent of 150 kg of N ha⁻¹ in liquid form in two parts i.e., before sowing and two weeks after sowing.

Three moisture treatments were used in the experiment. A schematic diagram explaining experiment in respect of treatments and sampling is given in Fig. 3.2. Non-stress (soil was kept at 70% drained upper limit), S6L: Stress initiated at the 6-leaf stage (water withheld at stage when 50% of the plants in the experiment showed the sixth leaf

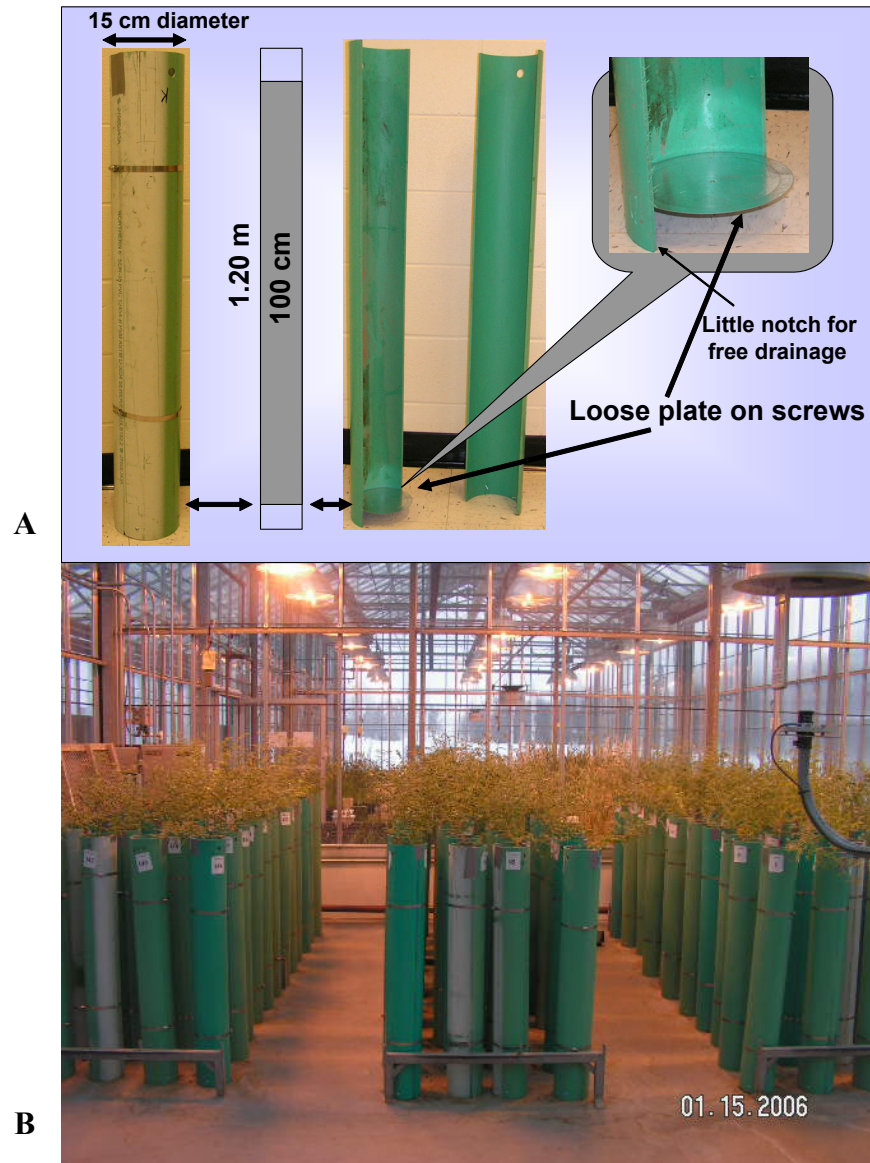


Fig. 3. 1: (A) Schematic diagram showing construction of cylinders, (B) A view of the experiment showing cylinders used for root collection in the greenhouse.

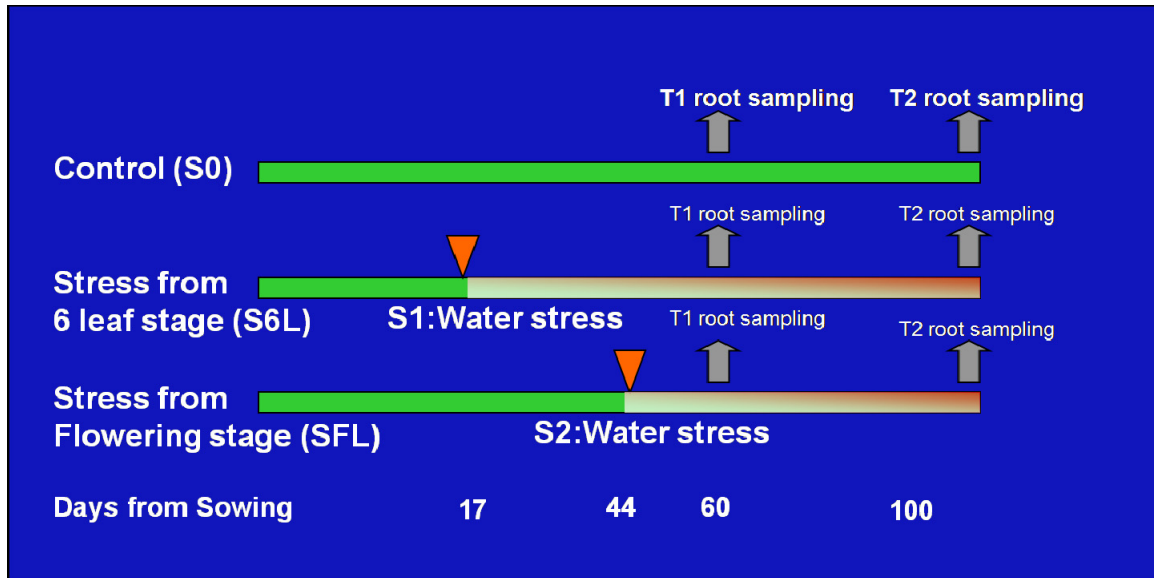


Fig. 3. 2: A schematic diagram showing treatment and root sampling scheme in the experiment.

fully expanded), SFL: Stress initiated at flowering (water withheld when 50% of the plants in the experiment were at the first flower stage). The cylinders were watered to field capacity two days before sowing. After emergence, plants were maintained near 70% of field capacity (determined by weight of the cylinders on alternate days) until the start of stress treatments, where plants were allowed to grow on progressively depleted soil moisture. The control treatment was kept near 70% of drained upper limit. The water requirements of the plants were determined as the daily difference between the weight of a fully irrigated cylinder and the weight of the cylinder 24 hours later, after the day's evapotranspiration. This determination was continued until the start of stress to account for the changing water demands of the plants with age.

Cylinders were placed in the greenhouse using a randomized complete block design in a split-split-plot arrangement with water treatments as main plots, growth stages

for root sampling as the sub-plot and genotypes as the sub-sub plot. All units were replicated three times and randomized at all levels. Temperature in the greenhouse was programmed for 25/18 °C day/night and photoperiod of 16/8 hours day/night regime with light supplemented with 400 W high-pressure sodium lights having photon flux density of 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a relative humidity of 60/70 % (day/night). Greenhouse control programs were kept the same in both trials.

Roots were sampled at two growth stages. These were designated as GS1, for two weeks after the appearance of first flower on 50% of the plants, and GS2, physiological maturity. Shoots were harvested and dry weights were recorded after drying in a hot air dryer at 45 °C for three days. To collect roots, each cylinder was opened longitudinally from one side. The soil core was sectioned into 20 cm lengths and each section was kept in sealable plastic bags which were stored at 5°C prior to root washing. Each section was washed carefully using 2 mm mesh sieves. Root data in each 20 cm section were recorded using a digital image analysis system (WinRhizo, Regent Instruments Inc., Canada). Special care was taken to avoid overlapping the roots or including soil debris. Soil volume for each section was also recorded and used to convert root length and weight into root length density and root weight density. In addition to the root data regarding length and weight, WinRhizo also produced other information on root diameter which is not discussed here due to the time limitations. After completing measurements with the digital image analysis system, root samples were dried at 80 °C for 72 hours and root dry weight was recorded.

Analysis of variance was performed for individual as well as combined trials using GenStat8 (Payne, 2006). The mean values of factors in the split- split- plot design

mode and their combinations along with their standard errors were computed. Soil depths were analyzed using repeated measures procedure of GenStat. Standard error for each depth was derived from analysis of individual depths.

3.4 Results

Significant genotypic variability was detected for various growth parameters among the eight chickpea genotypes assessed (Table 3.1). Root dry weight, shoot dry weight and root to shoot biomass ratio were significantly affected by treatments in both trials while root length showed significant treatment effects only in Trial I. Comparatively higher root and shoot biomass and root length was obtained in Trial I as compared to Trial II (Table 3.2 & 3.3).

3.4.1 Root dry weight and root weight density

Mean root dry weight in Trial I under the non-stress treatment was 2.37 g at flowering, and 2.59 g at maturity (Table 3.2). Mean root weight in Trial II under the non-stress treatment was 1.09 g at flowering, and 1.74 g at maturity. Mean root weight was reduced to 2.18 g at flowering and 1.89 g at maturity under the SFL treatment and further reduced to 1.80 g at flowering and 1.68 g at maturity under the S6L treatment in Trial I. Thus, total root dry weight decreased as drought stress intensity increased. Although root dry weights obtained in both trials are different, similar trends occurred in both trials. This difference in root weight between trials was likely due to the difference in the seasonal conditions. The weather outside the greenhouse during Trial II was mainly cloudy and with short winter days which affected the overall plant growth. In

Table 3. 1: Mean squares of growth parameters of eight chickpea genotypes grown under different moisture stress treatments in two trials during 2005-06.

	RDW (g) [¶]		RL (cm)		SDW (g)		RS Ratio	
	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
Treatment (T)	8.85*	25.91**	7.82*	2.86	220.50**	19.80**	14.68*	13.75*
Growth Stages (GS)	0.20	19.06**	2.76	1.19	22.22**	116.74**	31.08**	41.76**
Genotypes (G)	36.59**	33.99**	29.43**	22.35**	4.68**	5.95**	35.90**	30.18**
T x GS	0.96ns	21.87**	0.57	11.77**	12.48*	35.33**	1.73	0.69
T x G	2.17*	1.38	2.50**	0.92	1.31	1.39	1.33	1.66
GS x G	0.85	6.60**	1.28	4.15**	1.50	1.82	0.32	2.33*
T x GS x G	1.50	2.55**	1.81	2.28*	1.59	1.12	0.96	0.78

[¶]RDW: Root dry weight (g) per plant; RL: Root length (cm) per plant; SDW: Above-ground total shoot dry weight (g) per plant; RS Ratio: Ratio of root to shoot dry weight, Growth stages (GS) for root sampling. *, ** indicates significance at P=0.05, P=0.01, respectively.

Table 3. 2: Total root dry weight (g) and total root length (cm) per plant at flowering and maturity in a 100 cm soil profile for eight chickpea genotypes under different moisture stress treatments in two trials during 2005-06.

Genotype	Root Dry Weight (g)						Root Length (cm)					
	At Flowering [†]			At Maturity			At Flowering			At Maturity		
	S0 [¶]	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L
Trial I												
ILC 588	1.57	1.80	1.29	1.25	1.04	1.10	5640	5570	3970	3610	3380	3410
ILC 3182	0.76	1.60	1.61	2.08	1.47	0.95	1650	4570	4860	5570	4770	2840
ICCV 2	1.17	1.01	0.38	1.29	0.62	0.64	3010	2530	2060	4440	2380	2600
Amit	3.25	3.26	3.27	4.04	2.40	2.13	10270	7940	7420	9810	7180	6700
ILC 3279	3.29	2.61	2.28	3.37	2.22	2.58	8910	6480	4240	6640	5200	6010
ILC 10606	4.01	3.13	1.19	4.25	2.86	2.40	11880	8100	4210	10460	7210	7020
ILC 9955	3.37	2.98	3.16	3.09	3.34	2.13	8550	7670	7870	6840	6920	5120
CDC Chico	1.57	1.03	1.24	1.35	1.17	1.53	4510	2490	4010	2080	3430	4570
Mean	2.37	2.18	1.80	2.59	1.89	1.68	6800	5730	4830	6180	5060	4780
Se (+/-)	0.34 [£] (0.34)			0.34 (0.34)			827.5 (851.8)			827.5 (851.8)		
Trial II												
ILC 588	1.06	1.08	1.05	1.16	0.79	0.94	5020	4120	4950	5010	3630	3610
ILC 3182	0.92	1.52	1.17	1.13	0.99	1.04	3950	6130	5720	4820	4300	3590
ICCV 2	0.79	0.81	0.51	0.65	0.59	0.48	3410	3310	2180	2220	2830	1970
Amit	1.31	1.75	1.23	2.85	1.35	1.57	5680	7880	5480	10400	5910	5750
ILC 3279	1.70	2.29	1.56	2.41	2.17	1.84	6360	7910	6950	7180	6820	6440
ILC 10606	1.30	1.02	1.33	2.34	1.76	1.73	5150	4470	5090	6650	7350	7320
ILC 9955	0.58	1.70	1.38	2.69	1.85	1.55	2770	6680	5570	8740	6820	5430
CDC Chico	1.06	0.90	0.76	0.67	0.66	0.63	4730	3870	3520	2530	2970	2920
Mean	1.09	1.38	1.12	1.74	1.27	1.22	4630	5550	4930	5940	5080	4630
Se (+/-)	0.19 (0.19)						778.8 (786.8)			778.8 (786.8)		

[†]Growth stages: Flowering: sampled at flowering, Maturity: sampled at maturity

[¶]S0: Non-stress; SFL: Water withheld from flowering; S6L: Water withheld from the 6-leaf stage

[£] Standard error to compare the means within each level of moisture; Values in parenthesis are standard errors to compare means across moisture levels within a growth stage.

Table 3. 3: Shoot dry weight (g) per plant and root to shoot dry weight ratio at flowering and maturity for eight chickpea genotypes under different moisture stress treatments in two trials during 2005-06.

Genotype	Shoot Dry Weight (g)						Root to Shoot Dry Weight Ratio					
	At Flowering [†]			At Maturity			At Flowering			At Maturity		
	S0 [¶]	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L
Trial I												
ILC 588	9.37	10.01	7.60	14.71	8.52	7.19	0.16	0.18	0.19	0.08	0.12	0.14
ILC 3182	4.01	7.38	9.03	16.46	7.06	6.70	0.18	0.23	0.18	0.13	0.16	0.14
ICCV 2	5.87	4.82	2.61	12.43	7.07	6.09	0.18	0.19	0.17	0.10	0.10	0.07
Amit	9.00	8.79	8.56	17.74	8.59	6.61	0.36	0.31	0.38	0.27	0.29	0.32
ILC 3279	9.40	8.51	5.52	12.96	10.61	7.19	0.35	0.31	0.43	0.27	0.22	0.36
ILC 10606	9.55	8.77	3.52	17.15	8.34	5.81	0.46	0.37	0.31	0.28	0.34	0.42
ILC 9955	10.43	8.53	8.75	14.18	10.09	7.22	0.34	0.36	0.36	0.22	0.33	0.30
CDC Chico	10.10	8.13	4.74	19.70	10.37	9.19	0.20	0.15	0.28	0.09	0.12	0.16
Mean	8.47	8.12	6.29	15.97	8.83	7.00	0.28	0.26	0.29	0.17	0.21	0.24
Se (+/-)	1.5 [£] (1.4)			1.5 (1.4)			0.04 (0.04)			0.04 (0.04)		
Trial II												
ILC 588	5.50	5.99	4.07	11.35	7.54	5.60	0.20	0.18	0.26	0.10	0.11	0.17
ILC 3182	4.79	6.62	5.35	9.68	7.12	6.50	0.18	0.23	0.24	0.12	0.14	0.16
ICCV 2	4.88	6.32	3.93	7.14	6.85	3.80	0.16	0.14	0.13	0.10	0.08	0.12
Amit	4.41	5.51	3.23	10.24	7.18	4.51	0.29	0.33	0.38	0.30	0.19	0.36
ILC 3279	5.37	6.98	4.35	9.07	7.05	5.87	0.31	0.34	0.40	0.27	0.31	0.31
ILC 10606	4.57	4.34	3.01	8.62	5.91	4.67	0.28	0.24	0.41	0.29	0.31	0.38
ILC 9955	2.65	6.56	2.94	9.96	5.70	4.04	0.19	0.26	0.46	0.27	0.35	0.41
CDC Chico	4.95	4.77	3.77	8.80	6.76	4.86	0.22	0.19	0.21	0.08	0.09	0.13
Mean	4.64	5.89	3.83	9.36	6.76	4.98	0.23	0.24	0.31	0.19	0.20	0.26
Se (+/-)	0.63 (0.67)			0.63 (0.67)			0.04 (0.04)			0.04 (0.04)		

[†] Growth stages: Flowering: sampled at flowering, Maturity: sampled at maturity

[¶] S0: Non-stress; SFL: Water withheld from flowering; S6L: Water withheld from the 6-leaf stage

[£] Standard error to compare the means within each moisture level; Values in parenthesis are standard errors to compare means across moisture levels within a growth stage.

comparison, during Trial I, the weather outside the greenhouse was mainly sunny with long summer days.

Genotypes showed significant variation for root dry weight and this trait was also influenced significantly by the moisture stress conditions. Interactions among treatment and genotypes was significant in Trial I, but were not significant in Trial II. ILC 10606 produced the greatest root biomass along the 100 cm soil profile at flowering and maturity under the non-stress treatment in Trial I. ILC 3279 produced the greatest root biomass under the non-stress treatment at flowering stage, and Amit at maturity in Trial II. Amit produced the greatest root biomass under both stress treatments at the flowering stage, while ILC 9955 produced the greatest root biomass under SFL treatment, and ILC 3279 under S6L treatment at maturity in Trial I. ILC 3279 also produced the greatest root biomass in Trial II under both stress treatments at both growth stages.

Genotypes ICCV 2 and ILC 3182 produced the least root biomass under the non-stress treatment at flowering, while ICCV 2 and ILC 588 produced the least root biomass at maturity in Trial I. ICCV 2 produced the least root biomass under the non-stress treatment at flowering stage, while ICCV 2 and CDC Chico produced the least root biomass at maturity stage in Trial II. ICCV 2 also produced the least root biomass under both stress treatments at both growth stages in both trials. The least root biomass was produced under S6L compared to SFL. Generally, root weight decreased with increasing intensity of stress, but the magnitude of reduction differed among these genotypes. Genotypes ILC 10606, ILC 3279, Amit and ILC 9955 were able to produce higher root biomass under both stress treatments as compared to the other genotypes (ICCV 2, ILC 588, ILC 3182 and CDC Chico) (Table 3.2).

Water deficit also affected the distribution of root weight density (RWD) at various soil depths (Fig. 3.3). A significant reduction in root weight density for all the genotypes in both trials in the 0-20 cm layer was evident in the SFL treatment as compared to non-stress and was further reduced under the S6L treatment where stress was greater. Significant changes occurred for RWD in the 0-20 cm and 80-100 cm soil layers due to water deficit. RWD decreased significantly in the 0-20 cm soil layer while increased in the 80-100 cm layer. Under the non-stress treatment, 50% of the root dry weight was found in the 0-40 cm soil layer, while under both stress treatments, 50% of the root dry weight was found in 0-60 cm layer. This means that under water stress root biomass increased in the deeper soil layers to extract more water.

3.4.2 Root Length and Root Length Density

Highly significant genotypic variability was found for root length in both trials. The genotype by treatment interaction was significant during Trial I, but not significant in Trial II (Table 3.1). The greatest total root length along the 100 cm soil profile was produced by ILC 10606 and Amit at flowering and maturity in Trial I. Genotypes ILC 3279, Amit and ILC 10606 produced the greatest total root length at flowering, while Amit, ILC 9955 and ILC 3279 produced the greatest total root length at maturity in Trial II. Amit, ILC 3279, ILC 10606 and ILC 9955 also showed the greatest total root length under both stress treatments at flowering and maturity in both trials (Table 3.2).

Stress also affected the distribution of root length density (RLD) at different layers of the soil (Fig. 3.4). Significant reduction in RLD in the 0-20 cm layer was evident for all the genotypes in both trials under the SFL treatment as compared to the

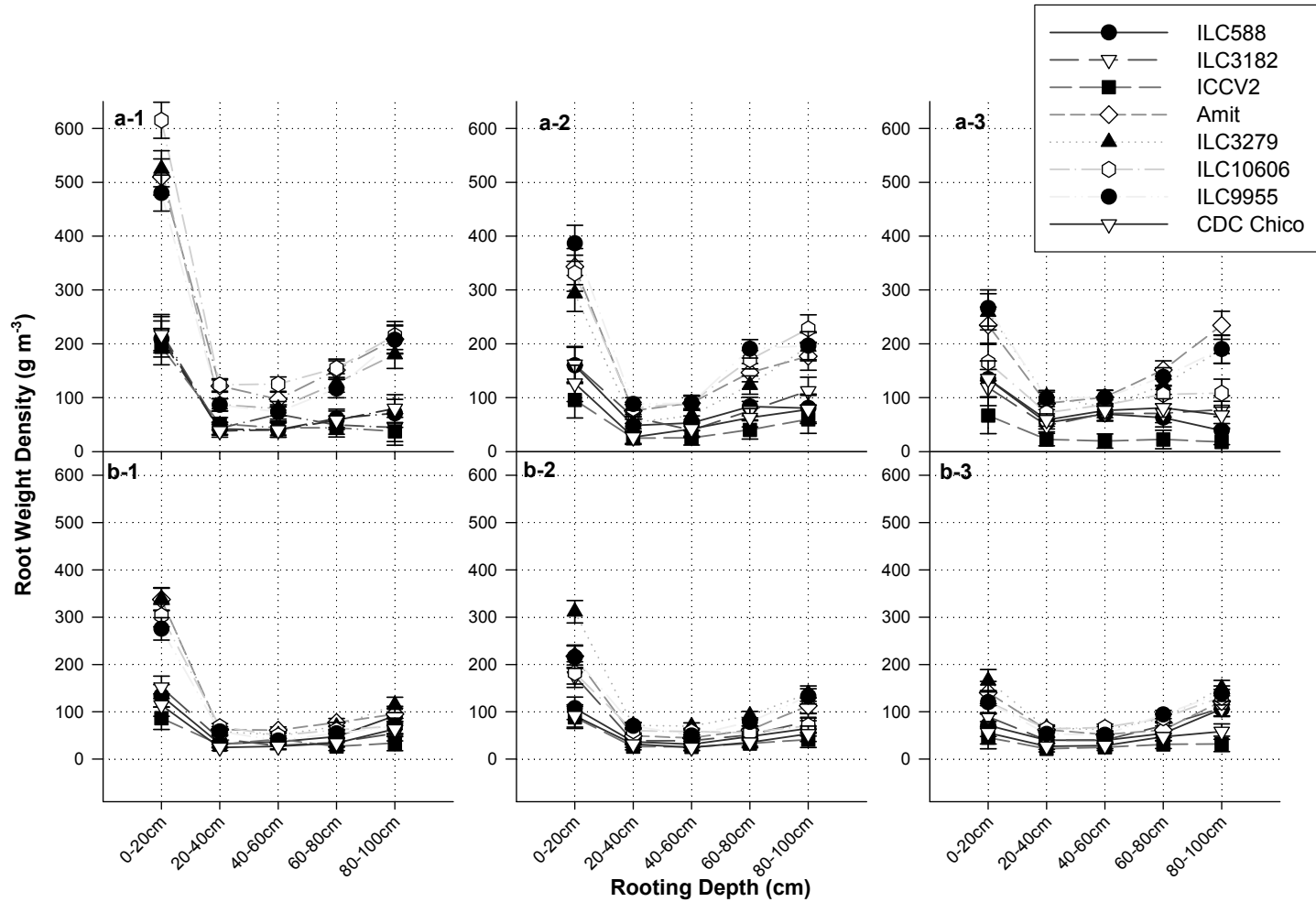


Fig. 3: Root weight density (g m^{-3}) distribution in different soil layers up to 100 cm depth for eight chickpea genotypes under non-stress (a1, b1), stress from flowering (a2, b2) and stress from 6 leaf stage (a3, b3) in Trial I (a) and Trial II (b), respectively during 2005-06. The error bars indicate standard errors (+/-) for each depth. Each data point represents the mean over 2 growth stages and 3 replications.

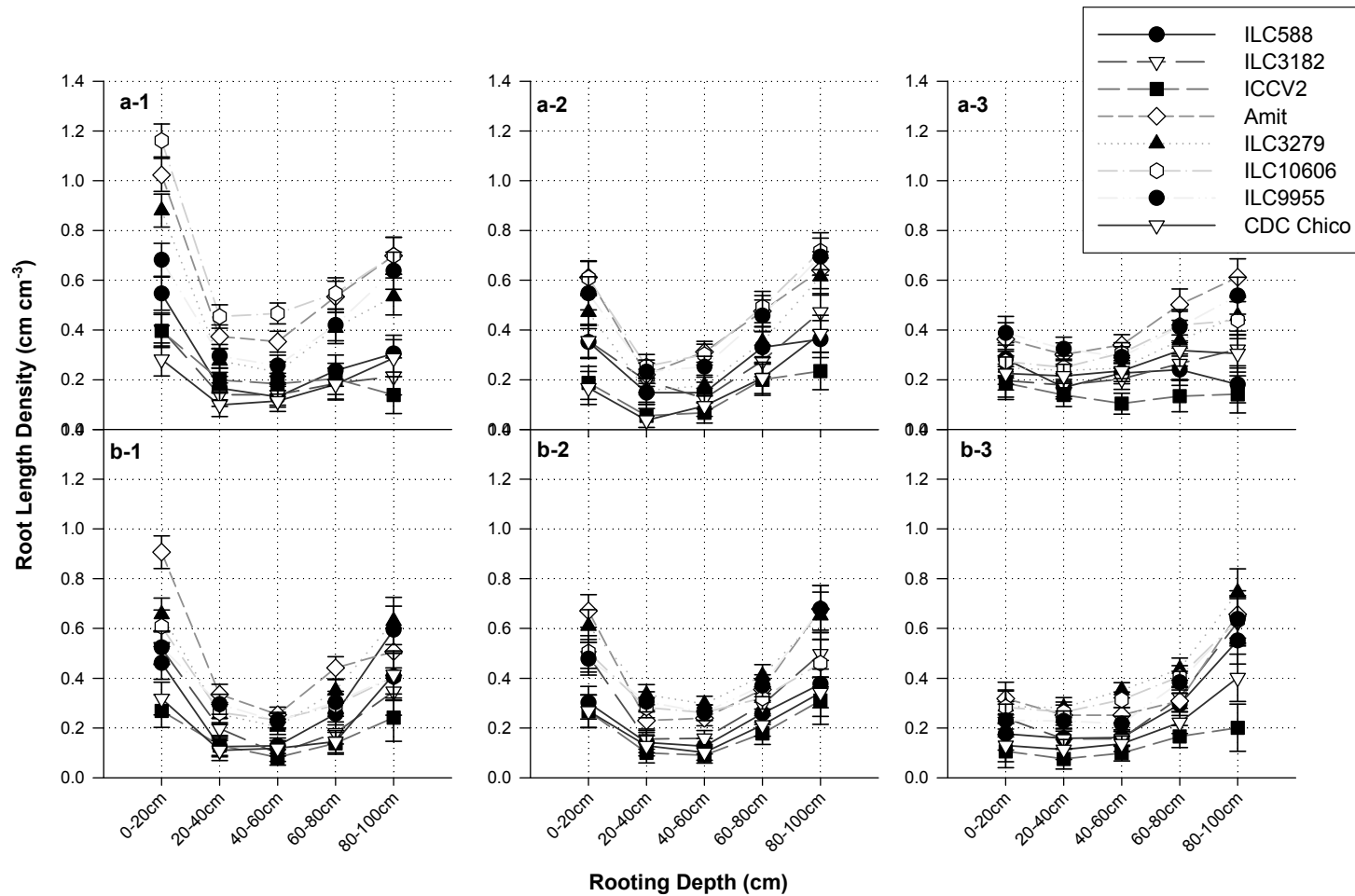


Fig. 3. 4: Root length density (cm cm^{-3}) distribution in different soil layers up to 100 cm depth for eight chickpea genotypes under non-stress (a1, b1), stress from flowering (a2, b2) and stress from 6 leaf stage (a3, b3) in Trial I (a) and Trial II (b), respectively during 2005-06. The error bars indicate standard errors (+/-) for each depth. Each data point represents the mean over 2 growth stages and 3 replications.

non-stress treatment and further reduced under the S6L treatment where stress was greater. Significant changes occurred for RLD in the 0-20 cm and 80-100 cm soil layers due to water deficit similar to RWD. RLD decreased significantly in the 0-20 cm soil layer due to the water deficit and increased slightly in the 80-100 cm layer.

About 50% of the root length was found in the 0-40 cm soil layer in the non-stress treatment, but under both stress treatments, 50% of the root length was found in the 0-80 cm layer. This implies that chickpea increased its root length in the deeper soil layer under stress to extract more water. A comparison of the rooting pattern of ILC 3279 and ILC 588 under stress and non-stress conditions is shown in Fig. 3.5. ILC 3279 maintained higher RLD in every soil layer under all three moisture treatments. A mapping population of recombinant inbred lines involving these two genotypes as parents has been developed and tested for various drought tolerance traits under drought environments (Chapter 5).

3.4.3 Shoot Dry Weight

Highly significant genotypic variation was found among genotypes studied for above ground total shoot dry weight (Table 3.1). Stress treatment effects were also highly significant. Interaction effects between stress treatment and genotypes were non-significant. Total shoot dry weight reduced as the drought stress increased in both the trials (Table 3.3).

3.4.4 Root to shoot weight ratio

Highly significant genotypic variation was found for root to shoot weight ratio (RS ratio) in both trials. Treatment effects were also significant while genotype by

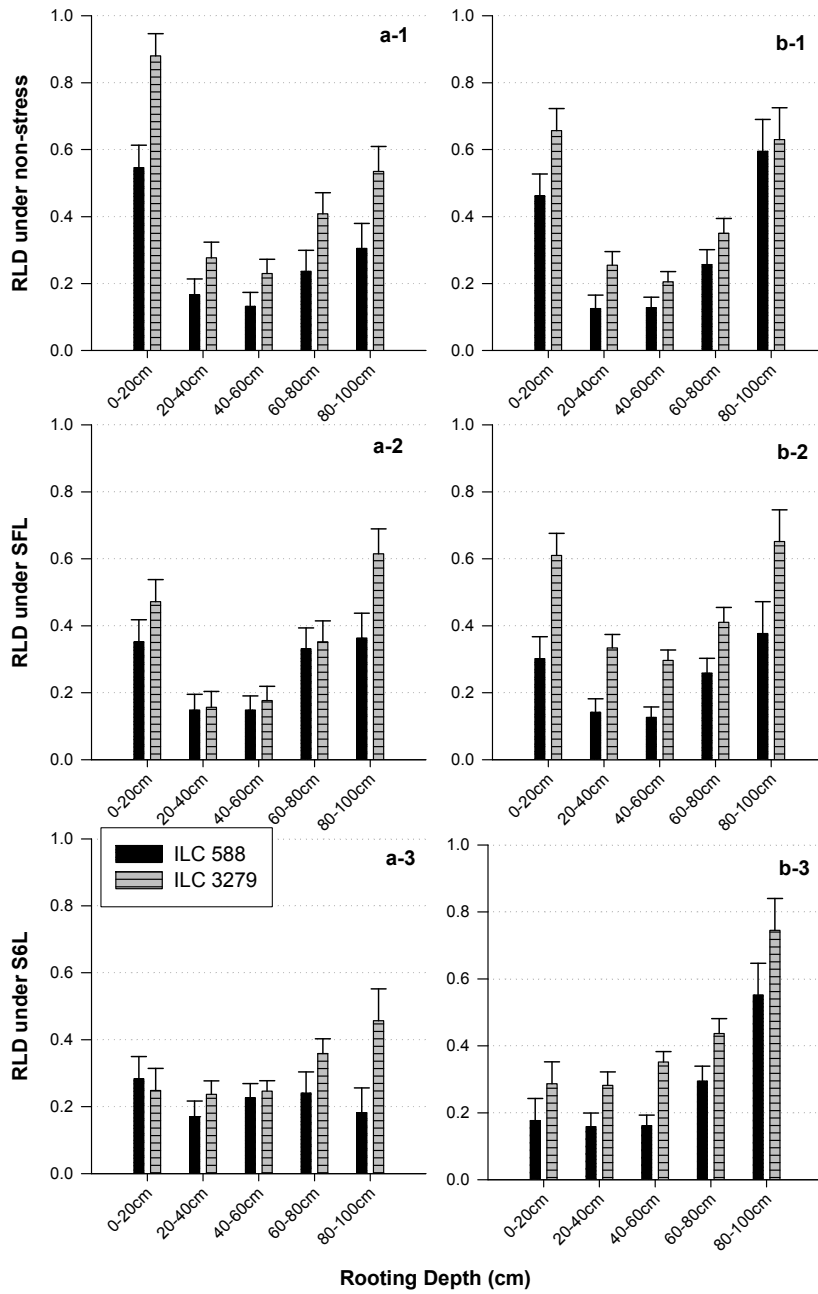


Fig. 3. 5: Comparison of root length density (cm cm^{-3}) of ILC 3279 and ILC 588 as affected by non-stress (1), stress from the 6 leaf stage (2) and stress from flowering (3) treatments in a 100 cm soil profile in Trial I (a) and Trial II (b) during 2005-06. The error bars indicate standard errors (+/-) for each depth. Each data point represents the mean over 2 growth stages and 3 replications.

treatment interactions were non-significant in both trials (Table 3.1). Mean root to shoot weight ratio obtained under the non-stress treatment was 0.28 (at flowering) and 0.17 (at maturity) in Trial I, and 0.23 (at flowering) and 0.19 (at maturity) in Trial II. Mean root to shoot weight ratio increased as drought stress intensity increased. Ali *et al.* (2005) also reported lower root to shoot weight ratio for chickpea genotypes under irrigated as compared to stress conditions. Amit, ILC 3279, ILC 1006 and ILC 9955 produced higher RS ratio under all the treatments in both trials as compared to ILC 588, ILC 3182, ICCV 2 and CDC Chico.

3.4.5 Root length to weight ratio

Root length to root weight ratio (LWR) is another important parameter to observe the changes in root densities. A significantly lower LWR was found in the 0-20 cm layer and the ratio increased as the depth increased for all the genotypes in both trials (Fig. 3.6). LWR was also lower in the 0-20 cm depth under moisture stress treatments as compared to the non-stress treatment, which may be due to the decay of fine roots in drying soil in the upper layer.

The mean numbers of days from sowing to flowering of genotypes having relatively smaller RLD (ILC 588, ILC 3182, ICCV 2 and CDC Chico) and higher RLD (ILC 3279, ILC 9955, ILC 10606 and Amit) were analyzed. The non-stress, SFL and S6L treatments took 41, 40 and 33 days in Trial I and 39, 39 and 39 days in Trial II, respectively in the case of genotypes with smaller root biomass. In contrast, genotypes having higher root biomass took 51, 51 and 52 days from sowing to flowering in Trial I

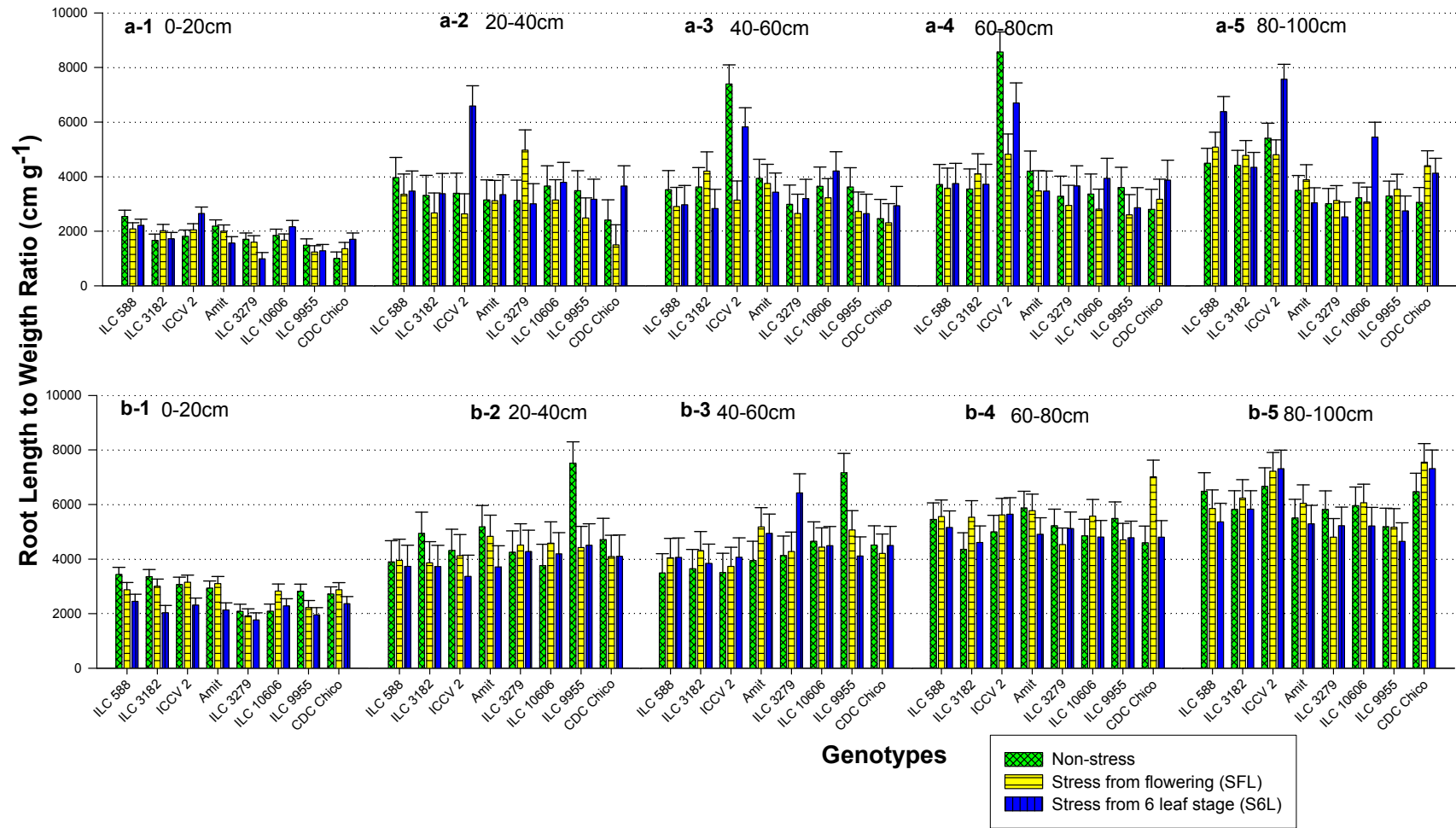


Fig. 3. 6: Root length to weight ratio (cm g^{-1}) distribution in different soil layers up to 100 cm depth under different stress treatments in trial I (a-1 to a-5) and trial II (b-1 to b-5) during 2005-06. The error bars indicate standard errors (+/-) for each depth. Each data point represents the mean over 2 growth stages and 3 replications.

and 66, 61 and 68 days in Trial II under non-stress, SFL and S6L treatments, respectively. For the total plant life cycle, the number of days from sowing to maturity in the genotypes with smaller biomass was 103, 85 and 75 in Trial I, and 91, 83 and 85 in Trial II under non-stress, SFL and S6L treatments, respectively.

Genotypes with greater root biomass had mean number of days from sowing to maturity of 107, 88 and 93 in Trial I and 99, 96 and 100 in Trial II under non-stress, SFL and S6L treatments, respectively. This seems that genotypes with higher root biomass have delayed maturity.

3.5 Discussion

The variations observed for root traits among genotypes due to water deficit were significant. Water deficit also affected the distribution of root weight density (RWD) and root length density (RLD) at various depths (Fig. 3.3 & 3.4). A significant reduction in RWD and RLD in the 0-20 cm layer was evident in the SFL treatment (where stress was initiated at flowering) compared to the non-stress treatment, and was further reduced under the S6L treatment (where stress was initiated at the 6-leaf stage) which had greater stress. Although overall root growth was reduced by stress, genotypes maintained or even increased their RWD and RLD into deeper soil layers (80-100 cm) in response to drought stress. Some of this increase could be attributed to the collection of roots at the bottom of the tube as curling of fine roots were observed at the bottom of the tube. This indicated that roots of chickpea could go more than 100 cm depth if they find the space to grow.

Under non-stress, about 50% of the root dry weight was found in the 0-40cm soil layer, while under both stress treatments, about 50% of the root dry weight was found in

the 0-60cm layer. This implies that root biomass increased in the deeper layers to extract more water.

In this research, RLD tended to increase in the 80-100 cm soil layer under both stress treatments as compared to non-stress. Chickpea genotypes, therefore, increased their water absorption capacity in deeper soil layer to cope with drought. Ali *et al.* (2005) also reported similar results. Total RLD under all the three treatments obtained in this study was higher in every soil layer as compared to field study under stress conducted by Kashiwagi *et al.* (2006). This was most likely due to the low and uniform soil bulk density along all soil layers in PVC cylinders used in our study as compared to field studies where soil bulk density varied and increased with soil depth.

A comparison of the rooting pattern of ILC 3279 and ILC 588 under stress and non-stress treatments (Fig. 3.5) demonstrated that genotypes maintained their relative ranking under varying moisture stress. Ali *et al.* (2005) also found that genotypic differences in root proliferation were maintained across stress conditions and growth media. This means that a genotype selected for superior rooting under one condition can maintain its superiority in other condition.

Increase in root to shoot weight ratio under both stress treatments was observed as compared to the non-stress treatment, primarily due to the relatively greater reduction in shoot biomass under drought stress conditions as compared to reduction in root biomass. Genotypes ILC 3279, ILC 955, ILC 10606 and Amit produced the greatest root to shoot weight ratio under all moisture treatments at both growth stages in both trials as compared to genotypes ICCV 2, ILC 588, ILC 3182 and CDC Chico.

Root length to root weight ratio (LWR) at various depths explains changes in root densities in response to moisture stress. A significantly lower ratio was found in the 0-20 cm layer and the ratio increased as the depth increased (Fig. 3.6). LWR was also lower in the 0-20 cm depth under moisture stress treatments as compared to non-stress treatment which might be due to the decay of fine roots in drying soil in the upper layer. Krishnamurthy *et al.* (1998) also found a substantial reduction in LWR in the 0-10 cm soil layer in the field during a dry season. LWR in the 0-20 cm layer under the non-stress treatment was also significantly lower than at deeper layers due to secondary thickening. Hence, less LWR in upper layers under moisture stress could be attributed to both, decay from drying soil and secondary thickening. LWR increased in deeper layers implying that roots became finer in the deeper soil layers. Fine roots with increased LWR are associated with increased water absorption, and are less prevalent in drier soils at all stages of crop growth (Krishnamurthy *et al.*, 1998). Stress effects on LWR were significant in Trial I only. Genotypes which produced relatively lower RWD or RLD (ILC 588, ILC 3182, ICCV 2 and CDC Chico) showed higher LWR in Trial I and similar LWR in Trial II compared with genotypes having significantly higher RWD or RLD (ILC 3279, ILC 10606, ILC 9955 & Amit). Therefore, genotypes ILC 588, ILC 3182, ICCV 2 and CDC Chico had comparatively finer root system or roots with lower specific density as compared to other genotypes. Ali *et al.* (2005) found non-significant effects of stress treatment on LWR. LWR may also be greatly affected by increasing soil bulk density with depth under field conditions.

Higher RLD and RWD had implications on time to flowering and maturity in this study (Table 3.4). Total RLD and total RWD had significant positive correlation with

Table 3. 4: Phenotypic correlation coefficients of root traits with days to flowering (DF) and days to maturity (DM) under contrasting moisture conditions in two trials during 2005-06.

	<u>DF</u> Under non-stress Condition	<u>DM</u>	<u>DF</u> Under SFL condition	<u>DM</u>	<u>DF</u> Under S6L condition	<u>DM</u>
Trial I						
RLDtot [¶]	0.77*	0.34	0.85**	0.22	0.87**	0.81*
RWDtot	0.71*	0.42	0.80**	0.18	0.80*	0.75*
Trial II						
RLDtot	0.73*	0.88**	0.93**	0.88**	0.79*	0.78*
RWDtot	0.86**	0.85**	0.89**	0.82*	0.85**	0.83*

[¶]RLDtot: Total root length density (cm cm⁻³); RWDtot: Total root weight density (g cm⁻³) along 100 cm soil profile.

SFL: Stress started from flowering stage; S6L: Stress started from the 6-leaf stage

*, ** indicates significance at P=0.05, P=0.01, respectively.

days to flowering and days to maturity, indicating that higher root densities might continue taking up water for longer period and delay maturation process. Genotypes with relatively smaller root densities might get drought signal earlier than other group of genotypes and help start the maturation process. Water deficiency also reduced root and shoot biomass, but reduction in shoot biomass was greater than root biomass, demonstrated by greater root to shoot dry weight ratios under the stress treatments. Genotypes ILC 588, ILC 3182, ICCV 2 and CDC Chico had comparatively lower RWD and RLD, but had finer roots due to high LWR. We found water deficit effects on LWR only in the Trial I. Ali *et al.* (2005) also found a non significant effect of water deficit on LWR. However, differences in LWR are difficult to detect under field conditions where bulk density changes with depth. Comparative differences in genotypes in this study were most profound in RLD and root biomass. For screening drought stress tolerance, RLD may be a better trait as it takes into account the finer roots in deeper soil layers responsible for water absorption which might be missed when simply measuring root biomass. The deeper root profile also contributes comparatively little to total root biomass. Krishnamurthy *et al.* (1996) and Ali *et al.* (2005) suggested RLD as a good

selection trait. Genotypes differed in their capacity to respond to the water deficits. Amit, ILC 3279, ILC 10606 and ILC 9955 had greater root biomass and root length and produced the majority of their roots in deeper soil layers under water deficit. This could be an important attribute of a cultivar for the regions with frequent drought stress. These genotypes could be used as parents in order to incorporate larger and deeper root system traits, an asset for cultivation in drought prone chickpea areas. On the other hand, genotypes ILC 588, ILC 3182, ICCV 2 and CDC Chico have comparatively smaller RLD and RWD and mature early. This group may be best adapted to regions of short growing season such as western Canada where crop growth termination is usually required prior to fall frost.

4. Effect of water deficit on phenology, yield, stomatal conductance and canopy temperature in chickpea (*Cicer arietinum* L.)

4.1 Summary

The aim of this study was to investigate the behavior of eight chickpea (*Cicer arietinum* L.) genotypes in response to moisture stress and non-stress treatments and to determine the relationship of stomatal conductance (g_s) and canopy temperature with phenology, harvest index (HI), yield and yield stability under changing moisture conditions. Experiments were conducted with three moisture treatments in two consecutive trials during 2005-06 in the Agriculture greenhouse, University of Saskatchewan, Saskatoon, Canada (52° N, 106° W). Grain yield stability was estimated by the drought susceptibility index (DSI) derived from the yield difference between stress and non-stress treatments. Grain yield was strongly associated with HI under stress and non-stress treatments. Drought susceptibility index, HI and grain yield were compared with midday g_s and canopy temperature. No relationship of g_s was found with DSI, HI or yield under the non-stress treatment, but significant relationships were observed under stress treatments. Similarly, canopy temperature showed a strong association with these traits under the stress treatments but not under the non-stress treatment. Higher canopy temperatures were associated with lower g_s (R^2 ranged from 0.46 to 0.83 in both trials) under the stress treatments suggesting that g_s and canopy temperature can be used directly as selection criteria. Stomatal conductance and canopy temperature at one week after the start of flowering showed significant association with grain yield and HI as compared to earlier and later dates of measurement.

4.2 Introduction

Drought is the major abiotic stress limiting crop yields worldwide. Breeding efforts for improvement of drought tolerance in crop plants is primarily based on selection for grain yield under drought stress. Because of the variability in drought pattern from year to year, further progress may not be achieved by selecting solely for grain yield. As water is fundamental to almost all aspects of plant growth, plants are thought to have evolved numerous strategies for coping with limited water availability including changes in phenological developmental and physiological traits (Passioura, 1996; Ackerly *et al.*, 2000; Araus *et al.*, 2002). To overcome the low response to a direct selection for yield under drought conditions, substantial efforts have targeted the manipulation of morpho-physiological traits influencing drought resistance through escape, avoidance and / or tolerance mechanisms (Ludlow and Muchow, 1990; Blum, 1996).

Several physiological criteria for selecting resistant genotypes have been proposed and demonstrated in other crops. Ritchie *et al.* (1990) observed in wheat that the most drought resistant genotypes had greater stomatal conductance under water-stress conditions than the susceptible genotypes. Yield was improved under drought by higher stomatal conductance in wheat (Fischer *et al.*, 1998). González *et al.*, (1999) also found a strong association between barley yield under drought and higher stomatal conductance.

The time required for the measurement of g_s for large populations and weather sensitivities under field conditions like diurnal fluctuations, sensitivity to cloud and wind, are the limitations of using stomatal conductance as a selection criterion under field conditions. An alternative is assessment of canopy temperature which is faster to measure and has a strong association with stomatal conductance (Amani *et al.*, 1996; Fischer *et al.*, 1998).

Hence, measurement of canopy temperature using an infrared thermometer is considered as a rapid indirect measurement of stomatal conductance. Canopy temperature was considered to be effective in screening wheat (Blum *et al.*, 1982; Pinter *et al.*, 1990) and pearl millet (Singh and Kanemasu, 1983) genotypes for resistance to drought.

Chickpea is an important legume crop mainly grown in drought prone areas of the world. Hence, it will be worthwhile to study the physiological basis of yield improvement in chickpea under drought. The objective of this experiment was to characterize eight diverse chickpea genotypes for agronomic and physiological parameters related to drought under non-stress and water stress treatments under greenhouse conditions and identify key traits to use for characterization of an intraspecific chickpea population under natural drought stress conditions.

4.3 Materials and Methods

4.3.1 Plant material

The trials were conducted with 8 chickpea genotypes comprising ILC 588, ILC 3182, ICCV 2, ILC 3279, ILC 10606, ILC 9955, Amit and CDC Chico. Genotypes ILC 588, ILC 3182, ICCV 2 originated in India, while ILC 3279 and ILC 10606 in former USSR, ILC 9955 in Uzbekistan, CDC Chico in Canada, while Amit is cultivated in Canada but originated in Eastern Europe. Genotypes ILC588, ILC3182 and ILC 3279 have been used as parents in QTL mapping populations for drought and ascochyta resistance in different centers around the world, hence study of these genotypes for physiological traits could help to explain their response to drought stress.

4.3.2 Experimental procedure

The first trial (Trial I) was conducted during 2005 (July 13 – October 28, 2005) and was repeated (Trial II) during 2005-06 (November 23, 2005 – February 27, 2006) in the Agriculture greenhouse, University of Saskatchewan, Saskatoon, SK, Canada (52°N, 106°W).

Cylinders were filled with a 1:1 mixture of potting mix (Sunshine mix # 4, Sun Gro Horticulture Canada Ltd., which contains peat moss, perlite, major and minor nutrients, gypsum, dolomitic limestone) and sand. Three seeds were sown in each cylinder and thinned to one after emergence and seedling establishment. Seeds were treated with fungicides Carbathiin, Thiabendazole (Crown) and Metalaxyl (Apron FL) prior to sowing. Fertilizer (Plant-Prod® 20-20-20 plus micronutrients; Plant Products Company Ltd., Brampton, Ontario, Canada) was supplied to provide the equivalent of 150 kg of N ha⁻¹ in liquid form in two equal splits before sowing and two weeks after sowing. Three moisture treatments were used in the experiment, S0: Non-stress or control (cylinder soil was kept at 70% drained upper limit i.e. 70% of the pot saturated weight after 24 hours drainage), S6L: Stress initiated at the 6-leaf stage (water was withheld at stage when 50% of the plants in the experiment showed the sixth leaf fully expanded), SFL: Stress initiated at flowering (water was withheld when 50% of the plants in the experiment were at the first flower stage). The cylinders were watered to saturation level two days before sowing and allowed to drain 24 hours to determine the weight of saturated cylinder. After emergence, plants were maintained at 70% of the cylinder saturated weight (determined by weight of the cylinders on alternate days) until the start of stress treatments, where plants were allowed to grow on progressively receding soil moisture. The control treatment was kept at 70% of

the cylinder saturated weight. The water requirements of the plants were determined as the difference between the weight of a fully irrigated cylinder and the weight of the cylinder 24 hours later, after the day's evapotranspiration. This determination was conducted on alternate days to take care of changing water demands of the plants with age.

Cylinders were placed in the greenhouse within a randomized complete block design in a split-plot arrangement in three replications with water treatments as main-plots and genotypes as sub-plots. All experimental units were randomized at all levels. Temperature in the greenhouse was programmed for 25/18 °C day/night and photoperiod of 16/8 hours day/night regime under natural light supplemented with 400 W high-pressure sodium lights having photon flux density of 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a relative humidity of 60/70 % (day/night). The natural daylength declined from 16 hours to 10 hours over the course of Trial I, and increased from 8.5 hours to 9.5 hours over the course of Trial II. Shoots were harvested at physiological maturity and dry weights were recorded after drying in a hot air dryer at 45 °C for three days. Then shoots were threshed and weight of grain yield recorded on individual plants.

4.3.3 Measurement of stomatal conductance

Stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) was measured with a steady state porometer (Li-1600, LI-COR Inc., Lincoln, NE), with cuvette conditions set to ambient. The Li-Cor 1600 operates on the null balance principal. When the transpiring intact leaf is inserted into the cuvette (Fig. 2.4, chapter 2), it raises the humidity level inside the cuvette. To balance this increased humidity, an internal flow controller increases the flow of dry air to the cuvette until it balances the humidity to a predetermined set point which is ambient

RH. As different genotypes transpire at different rates, different amounts of dry air are required to reach the set point. This difference is used to compute the stomatal conductance. Measurements in both trials were recorded on the third from top fully expanded well-lit leaf around mid day (10.00 to 14.00) at weekly intervals starting from flowering. All the measurements were made on clear and sunny days.

4.3.4 Measurement of canopy temperature

A hand-held infrared thermometer (Everest Interscience Inc., Fullerton, CA) with 4° field of view, was used to measure canopy temperature (°C). The data for each plant were the mean of two readings, each of which was the average of 10 readings, taken from both sides of each unit, at an angle of approximately 30° to the horizontal, in a range of directions such that it shoot plant canopy. Special care was taken for the thermometer not to view other than plant canopy or leaves i.e. soil, floor or windows. Data were recorded around midday at weekly interval during both trials.

4.3.5 Statistical analysis

Yield stability, or the extent of variation in yield between stress and non-stress conditions, is widely accepted as an indicator of genotypic response to stress (Blum, 1988). Hence the ‘susceptibility index’ of Fischer and Maurer (1978), which is in accordance with the theory of Langer *et al.* (1979), was calculated in this study. This method involved testing all genotypes in only two environments which constitute stress and non-stress conditions for the environmental factor involved. The drought susceptibility index (DSI) estimates for each genotype as the rate of change in yield between the two environments

relative to the mean change for all the genotypes. Hence, DSI for two stress treatments (SFL & S6L) was calculated as follows:

$$DSI_{(\text{for SFL})} = (1 - Y_{\text{SFL}}/Y_P) / (1 - X_{\text{SFL}}/X_P) \quad [1]$$

$$DSI_{(\text{for S6L})} = (1 - Y_{\text{S6L}}/Y_P) / (1 - X_{\text{S6L}}/X_P) \quad [2]$$

Where Y_{SFL} & Y_{S6L} are yield under SFL and S6L stress treatments, respectively; Y_P is yield without stress, X_{SFL} & X_{S6L} represents average yield over all varieties under SFL & S6L stress treatments, respectively, and X_P represent average yield over all genotypes under the non-stress treatment. The term $(1 - X/X_P)$ is defined as ‘stress intensity’ (δ_i).

Harvest index (HI) was calculated according to the formula:

$$HI = \text{Grain weight} / \text{Total aboveground dry weight} \quad [3]$$

Analysis of variance was performed for all data based on a split-plot design using GenStat8 (Payne, 2006). Data for each date of measurements were analyzed individually. The mean values of factors along with their standard errors were computed. Relationships between parameters were determined using Pearson’s simple correlation test of GenStat8 (Payne, 2006)

4.4 Results

4.4.1 Phenology

Mean data and mean squares for days to flower and maturity are presented in Table 4.1 for the water stress and non-stress treatments for both trials. Genotypes showed significant differences for days to flowering and maturity in both trials. Drought treatment effects for days to flower were non-significant, but were significant for days to maturity in both trials. ILC 588, ILC 3182, CDC Chico and ICCV 2 were earlier in terms of days to flowering under stress and non-stress treatments as compared to ILC 3279, Amit, ILC 10606 and ILC 9955. This difference in terms of days to flowering between the above two groups of genotypes was greater during Trial II. Maturity generally occurred earlier in the water stress treatments than non-stress treatments for all the genotypes, resulting in a shortened grain-filling period. On average, genotypes matured 19 days earlier under the SFL treatment (where stress was initiated at the flowering stage) and 21 days earlier under the S6L treatment (where stress was initiated at the six leaf stage) as compared to the non-stress treatment (S0) in Trial I, and six days earlier under the SFL and three days earlier under the S6L in Trial II. ICCV 2 was the earliest maturing genotype and Amit was the latest in both trials with a difference of 12 days in Trial I and 17 days in Trial II under the S0 treatment. ICCV 2 matured eight days earlier under the SFL treatment and 27 days earlier under the S6L treatment compared to the S0 in Trial I, while in Trial II, ICCV 2 matured seven days earlier under the SFL than the S0 and one day later under the S6L treatment. Amit matured 16 days earlier under the SFL compared to the S0, and 13 days earlier under the S6L compared to the S0 during Trial I. In Trial II, Amit matured eight days earlier under the SFL and at the same time as S0 under the S6L treatment.

Table 4. 1: Mean data for days to flowering, days to maturity, grain yield per plant and harvest index, as well as mean squares for eight chickpea genotypes grown under different moisture stress conditions in two trials during 2005-06.

Genotype	<u>Days to flowering</u>			<u>Days to maturity</u>			<u>Grain Yield (g plant⁻¹)</u>			<u>Harvest Index</u>		
	S0 [¶]	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L
Trial I												
ILC 588	41	37	30	99	74	67	7.8	4.1	3.6	0.52	0.48	0.52
ILC 3182	40	44	41	105	89	85	8.3	3.0	2.8	0.50	0.42	0.41
ICCV 2	50	39	30	97	89	70	5.3	4.0	3.2	0.42	0.56	0.57
Amit	49	53	55	109	93	96	6.9	1.0	1.3	0.39	0.12	0.19
ILC 3279	50	41	47	103	79	90	2.9	3.0	0.7	0.27	0.28	0.10
ILC 10606	56	61	56	108	95	98	3.5	0.6	0.3	0.18	0.08	0.06
ILC 9955	46	50	49	106	84	87	3.9	0.7	0.9	0.27	0.08	0.11
CDC Chico	31	39	32	112	90	76	10.6	4.3	3.3	0.51	0.41	0.37
Mean	46	46	43	105	87	84	6.1	2.6	2.0	0.38	0.30	0.29
Se (+/-)	4.8(4.6) [£]			5.7(5.9)			1.0(1.1)			0.05(0.05)		
	<u>Days to flowering</u>			<u>Days to maturity</u>			<u>Grain Yield (g plant⁻¹)</u>			<u>Harvest Index</u>		
<i>Mean squares</i>												
Treatment(T)	73.47			3247.44**			120.13**			0.033*		
Genotype(G)	592.99**			395.77**			25.64**			0.258**		
T x G	73.54			107.00			4.53			0.014*		
Trial II												
ILC 588	40	37	37	97	86	83	5.3	3.3	2.4	0.46	0.44	0.44
ILC 3182	43	48	42	94	87	87	4.3	2.7	2.6	0.43	0.38	0.40
ICCV 2	33	34	37	84	77	85	4.2	3.8	1.9	0.59	0.55	0.52
Amit	61	56	63	101	93	101	1.3	1.5	1.1	0.12	0.21	0.18
ILC 3279	60	63	62	97	95	96	0.8	0.4	0.5	0.08	0.06	0.08
ILC 10606	76	64	72	101	99	102	0.3	0.1	0.2	0.03	0.02	0.04
ILC 9955	65	62	75	99	96	99	0.4	0.3	0.1	0.04	0.04	0.01
CDC Chico	42	39	39	91	82	84	4.6	2.9	2.1	0.52	0.43	0.43
Mean	53	50	53	95	89	92	2.6	1.9	1.4	0.28	0.27	0.26
Se (+/-)	3.2(3.3)			2.3(2.4)			0.3(0.3)			0.03(0.03)		
	<u>Days to flowering</u>			<u>Days to maturity</u>			<u>Grain Yield (g plant⁻¹)</u>			<u>Harvest Index</u>		
<i>Mean squares</i>												
Treatment(T)	58.55			216.32*			9.67*			0.003		
Genotype(G)	1830.34**			417.81**			20.57**			0.419**		
T x G	38.37			27.9			1.25**			0.002		

¶S0: Non-stress; SFL: Water withheld from flowering stage; S6L: Water withheld from 6 leaf stage

£ Standard error to compare the means within each level of moisture; Values in parenthesis are standard errors to compare means across moisture levels.

4.4.2 Yield and harvest index

Mean data and mean squares for grain yield (g) per plant and harvest index are presented in Table 4.1 for non-stress and water stress treatments for both trials. Mean squares for genotypes and treatments for grain yield were significant in both trials while their interaction was only significant in Trial II. Grain yield was greater under non-stress treatment than under water stress treatments for all genotypes in both trials. Mean grain yield over all the genotypes under non-stress treatment was 6.1 g per plant in Trial I and 2.6 g per plant in Trial II. Mean reductions in grain yield due to the SFL and S6L treatments were 58% and 67% in Trial I, and 29% and 48% in Trial II, respectively.

Mean squares for genotypes for HI were significant in both trials, while drought treatment effects and the genotype x treatment interaction effects were only significant in Trial I. Water stress effects on harvest index were only significant in Trial I where mean harvest index was reduced from 0.38 in S0 to 0.30 in the SFL treatment and 0.29 in the S6L treatment. Trial II showed the same pattern although differences were not significant.

CDC Chico, ILC 588, ILC 3182 and ICCV 2 produced the greatest grain yield under non-stress and both stress treatments in both trials. These genotypes also showed higher harvest index under non-stress and stress treatments in both trials. ICCV 2 had the greatest harvest index under the SFL and S6L in both trials followed by ILC 588, ILC 3182 and CDC Chico. Genotypes ILC 10606, ILC 9955, ILC 3279 and Amit had the least HI.

4.4.3 Drought susceptibility index

Drought susceptibility index (DSI) values for eight chickpea genotypes under both stress treatments during both trials are presented in Table 4.2. Genotypes ICCV2, ILC 588,

Table 4. 2: Drought susceptibility index (DSI) of eight chickpea genotypes grown under different moisture stress treatments in two trials during 2005-06.

Genotype	Drought Susceptibility Index (DSI)			
	Trial I		Trial II	
	<u>SFL</u> [¶]	<u>S6L</u>	<u>SFL</u>	<u>S6L</u>
ILC 588	0.8	0.8	1.1	1.3
ILC 3182	1.0	1.1	0.8	1.3
ICCV 2	0.6	0.4	1.1	0.3
Amit	1.2	1.5	0.3	-0.4
ILC 3279	1.1	-0.1	0.7	1.5
ILC 10606	1.3	1.4	0.4	1.6
ILC 9955	1.2	1.4	1.9	1.2
CDC Chico	1.0	1.0	1.1	1.3
Mean	1.0	1.0	0.9	1.0

¶SFL: Water withheld from flowering stage; S6L: Water withheld from 6 leaf stage

ILC 3182 and CDC Chico were relatively drought tolerant ($DSI \leq 1$) while genotypes ILC 10606, ILC 9955, Amit and ILC 3279 were relatively drought susceptible ($DSI > 1$) under the SFL treatment during Trial I. Similar results found under the S6L treatment with the exception of ILC 3279. Results obtained in the Trial II showed higher DSI values as compared to Trial I for all genotypes with the exception of Amit. Genotypes ILC 10606, ILC 9955 and ILC 3279 showed higher values of DSI than other genotypes under the S6L treatment.

4.4.4 Stomatal Conductance

Leaf stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) was measured at weekly interval at five dates starting from pre-flowering during Trial I and four dates during Trial II. Mean data over all dates for Trial I and Trial II are presented in Table 4.3 & 4.4, respectively. The first measurement date during Trial I was five weeks after sowing (WAS) where the majority of the genotypes started flowering during seven WAS and the first measurement

Table 4. 3: Mean stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) for various measurement dates during crop growth in Trial I and their correlations with grain yield, HI and drought susceptibility index (DSI) for eight chickpea genotypes.

Genotypes	5 WAS ¹			6 WAS			7 WAS			8 WAS			10 WAS		
	S0 [†]	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L
ILC 588	429	366	414	445	369	203	591	324	332	750	326	297	244	153	101
ILC 3182	529	366	362	438	384	236	450	239	351	467	267	160	193	201	40
ICCV 2	401	435	409	439	410	483	516	651	613	668	635	421	205	216	132
Amit	233	268	179	189	267	185	337	206	261	229	84	108	72	51	80
ILC 3279	465	314	248	473	340	257	502	196	193	546	129	78	229	25	21
ILC 10606	290	305	116	269	285	195	318	220	185	316	176	100	73	25	21
ILC 9955	406	380	340	378	398	362	289	121	224	248	59	131	137	27	69
CDC Chico	198	487	240	126	436	334	212	544	173	167	305	173	178	136	66
Mean	369	365	289	345	361	282	402	313	292	424	248	184	166	104	66
Se (+/-)	59.2(56.1) [‡]			57.6(56.7)			101.8(114.8)			107.2(112.8)			39.7(39.4)		
Correlations with:															
GY (g plant ⁻¹)	-0.28	0.67	0.68	-0.43	0.66	0.30	-0.16	0.73*	0.54	-0.14	0.71*	0.74*	0.20	0.75*	0.64
HI	0.06	0.63	0.76*	-0.03	0.64	0.39	0.25	0.76*	0.76*	0.27	0.86**	0.88**	0.52	0.90**	0.74*
DSI	NA	-0.18	-0.84**	NA	-0.29	-0.60	NA	-0.34	-0.85**	NA	-0.42	-0.94**	NA	-0.23	-0.80**

[†]S0: Non-stress; SFL: Water withheld from flowering stage; S6L: Water withheld from 6 leaf stage

¹WAS: weeks after sowing

[‡] Standard error to compare the means within each level of moisture; Values in parenthesis are standard errors to compare means across moisture levels.

Table 4. 4: Mean stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) for various measurement dates during crop growth in Trial II and their correlations with grain yield, HI and drought susceptibility index (DSI) for eight chickpea genotypes.

	8 WAS ¹			9 WAS			10 WAS			11 WAS		
	S0 [¶]	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L
ILC 588	349	339	283	622	484	418	183	146	104	124	18	24
ILC 3182	665	231	214	661	367	121	139	140	135	103	54	43
ICCV 2	662	462	199	917	513	542	237	120	221	169	29	171
Amit	273	270	254	716	261	406	161	119	143	64	31	87
ILC 3279	237	151	193	668	418	394	176	125	140	157	38	50
ILC 10606	480	252	343	852	482	467	217	138	109	158	68	84
ILC 9955	575	281	526	893	828	547	248	233	199	154	78	85
CDC Chico	731	600	465	1072	476	657	268	137	220	171	132	92
Mean	497	323	310	800	479	444	204	145	159	137	56	80
Se (+/-)	93.9(98.8) [£]			104.4(107.0)			30.2(37.7)			29.0(30.2)		
Correlations with:												
GY(g plant^{-1})	0.43	0.68	-0.31	0.01	-0.24	-0.03	-0.01	-0.40	0.27	-0.0	-0.09	0.21
HI	0.56	0.64	-0.35	0.20	-0.25	-0.30	0.14	-0.40	0.04	0.15	-0.14	-0.10
DSI	NA	-0.15	0.61	NA	0.38	0.40	NA	0.31	0.57	NA	0.37	0.13

[¶]S0: Non-stress; SFL: Water withheld from flowering stage; S6L: Water withheld from 6 leaf stage

¹WAS: weeks after sowing

[£] Standard error to compare the means within each level of moisture; Values in parenthesis are standard errors to compare means across moisture levels.

date during Trial II was eight WAS where the majority of the genotypes started flowering during eight WAS. None of the measurement dates except six WAS in Trial I showed significant genotype x treatment interactions in either trial. In S0, g_s was generally higher (difference $g_s \sim 100 \text{ mmol m}^{-2} \text{ s}^{-1}$) than in the S6L throughout the data collection period during both trials. The difference between S0 and SFL was least in first two measurements, but this difference was increased (difference $g_s > 100 \text{ mmol m}^{-2} \text{ s}^{-1}$) thereafter. In the SFL and S6L, stomatal conductance fell quite sharply ($g_s > 350 \text{ mmol m}^{-2} \text{ s}^{-1}$ at flowering while $g_s < 100 \text{ mmol m}^{-2} \text{ s}^{-1}$ at 10 WAS) during the experiment as the plants dehydrated. Stomatal conductance under the S6L declined more rapidly than under SFL. Regarding g_s response to plant age, g_s tended to be higher around flowering (g_s range 275 – 400 $\text{mmol m}^{-2} \text{ s}^{-1}$) and fell sharply as plants approached maturity (g_s range 66 – 100 $\text{mmol m}^{-2} \text{ s}^{-1}$). Weekly stomatal conductance of ILC 588 and ILC 3279 genotypes with contrasting response is shown in Fig. 4.1. Under both stress treatments as compared to the non-stress treatment, there was a steady decrease in g_s in ILC 588 (g_s range 100 – 150 $\text{mmol m}^{-2} \text{ s}^{-1}$ at 10 WAS under stress), while g_s in ILC 3279 were very low, and there was a sharp decrease as plants approached maturity (g_s range 20 – 25 $\text{mmol m}^{-2} \text{ s}^{-1}$ at 10 WAS under stress). ICCV 2, ILC 588, ILC 3182, ILC 9955 and CDC Chico generally had higher g_s under both stress and non-stress treatments than the other genotypes.

Correlation coefficients for g_s with grain yield, HI and DSI are presented in Table 4.3 and 4.4. No significant correlation was found for g_s from any measurement date with grain yield, HI and DSI under the S0 for both trials. However, the correlation coefficients g_s were significant under S6L during Trial I with grain yield ($r = 0.71/0.74$ under the SFL/S6L treatment at 8 WAS), HI ($r = 0.86/0.88$ under the SFL/S6L treatment at 8

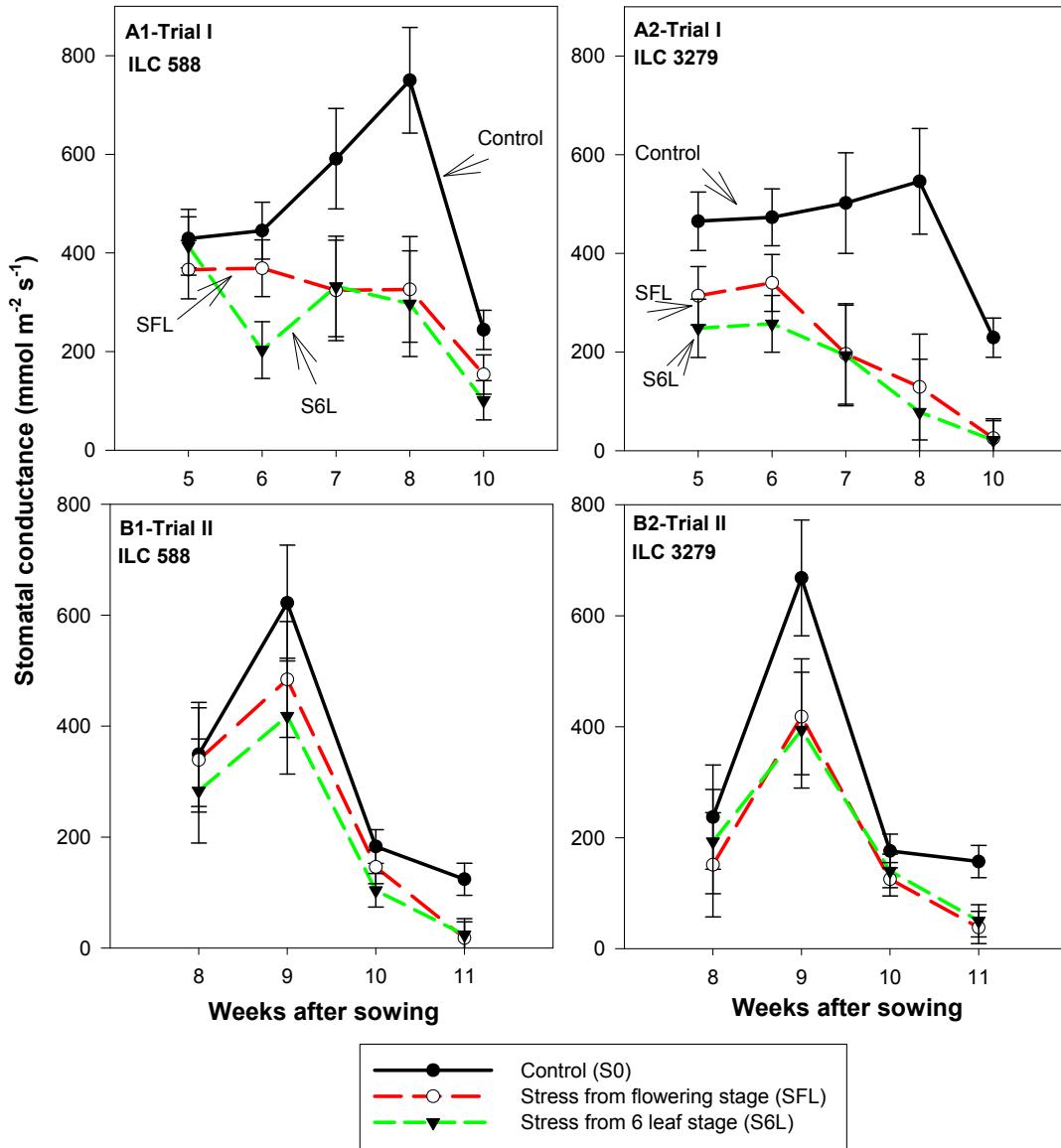


Fig. 4. 1: Stomatal conductance (mmol m⁻²s⁻¹) versus time under non-stress and water stress treatments in genotypes ILC 588 and ILC 3279 during Trial I (A) and Trial II (B). Vertical bars represent the standard errors (+/-).

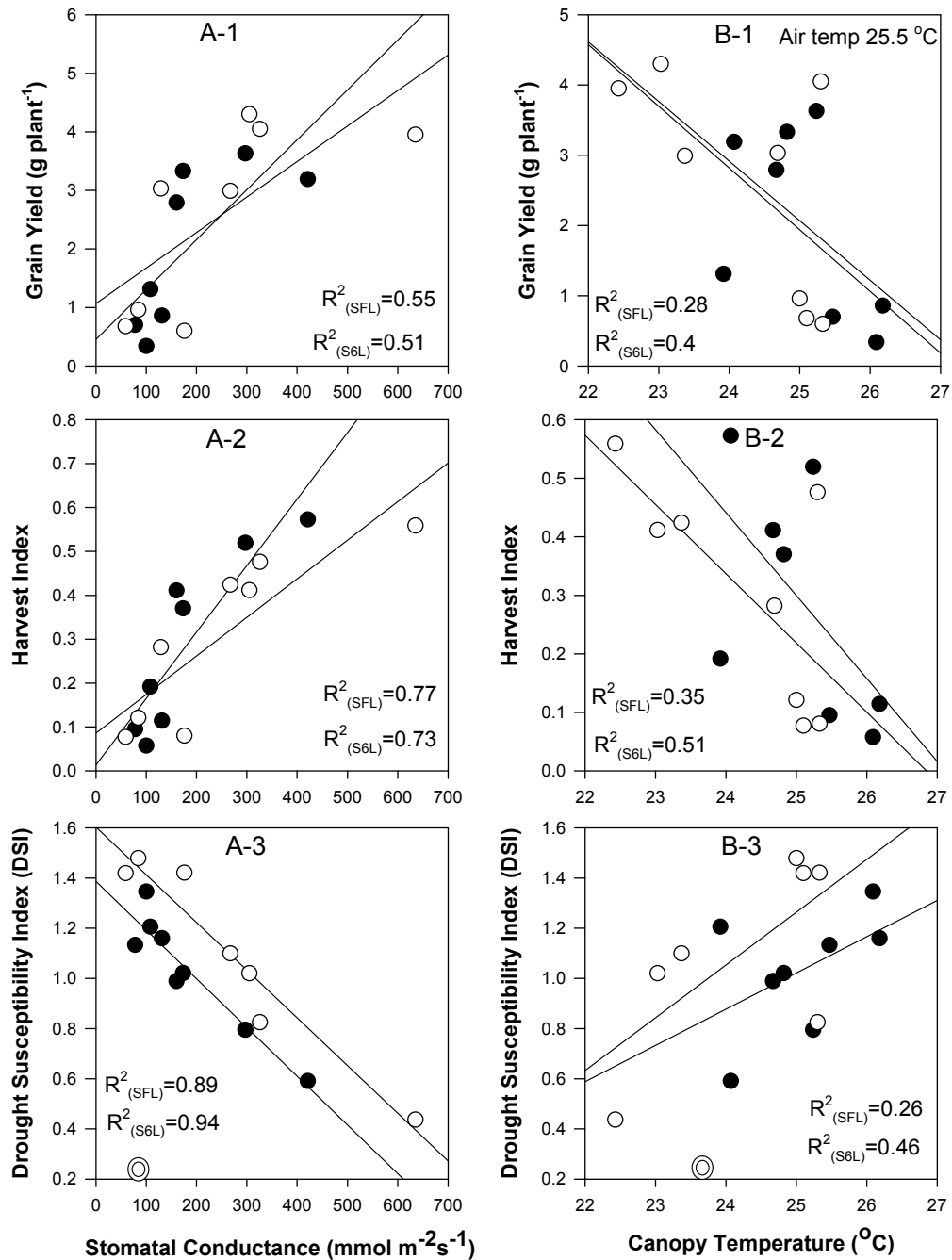


Fig. 4. 2: The relationship of stomatal conductance (A) and canopy temperature (B) measured on same day at 8 weeks after sowing (WAS) with grain yield (1), HI (2) and DSI (3) for eight chickpea genotypes evaluated during Trial I. Flowering was started during sixth week after sowing. Mean air temperature during canopy temperature measurement was 25.5 °C. Solid circles (●) represent data under SFL treatment while open circles (○) represent data under S6L treatment. The data points circled in graph A-3 and B-3 was excluded before regression analysis.

WAS) and DSI ($r = -0.42/-0.94$ under the SFL/S6L treatment at 8 WAS). On all measurement dates, a positive correlation was observed for g_s and grain yield, g_s and HI and a negative correlation for g_s with DSI. Correlations were more significant (r range 0.54 to 0.74 in case of g_s vs grain yield, 0.76 to 0.88 in case of g_s vs HI and -0.34 to -0.94 in case of g_s vs DSI) at the seven and eight WAS (both dates fell during flowering) as compared to earlier or later dates. Both these dates were during the flowering period. Correlation coefficients in Trial II were not significant under any treatment, which may have been due to the different environmental conditions outside the greenhouse at the time of measurements during Trial II. Measurements during Trial II were conducted during January while in Trial I, they were conducted during August - September. Despite utilizing the same light supplementation, day lengths were shorter during Trial II, and the occurrence of cloudy days were more frequent.

Linear regression of g_s and canopy temperature measured at eight WAS with grain yield, HI and DSI during the Trial I under both stress treatments are presented in Fig. 4.2. These graphs indicated that grain yield and HI can be increased through higher g_s . Stomatal conductance also showed a significant negative linear relationship with DSI ($R^2 = 0.89/0.94$ under the SFL/S6L treatment). Grain yield and HI under both drought stress treatments fell down severely as g_s was reduced from >250 to $150 \text{ mmol m}^{-2}\text{s}^{-1}$, and showed severe drought stress or yield penalty due to drought stress. Grain yield obtained in this range of g_s ($<150 \text{ mmol m}^{-2}\text{s}^{-1}$) was less than 1.3 g plant^{-1} while HI was less than 0.2. Stomatal conductance ranged from 150 to $250 \text{ mmol m}^{-2}\text{s}^{-1}$ showed partial drought stress and partial decreases in grain yield ($<3.2 \text{ g plant}^{-1}$) and HI (<0.4). Stomatal conductance values $>250 \text{ mmol m}^{-2}\text{s}^{-1}$ showed better grain yield ($>3 \text{ g plant}^{-1}$) and HI

(>0.4). A similar trend was found when g_s compared with DSI. Stomatal conductance values $<150 \text{ mmol m}^{-2}\text{s}^{-1}$ showed plants had higher drought susceptibility (DSI >1.1), while plants with g_s ranging from 150 to $250 \text{ mmol m}^{-2}\text{s}^{-1}$ showed plants had DSI values ranging from 0.8 to 1.1. Plants had higher g_s ($>250 \text{ mmol m}^{-2}\text{s}^{-1}$) showed lower drought susceptibility (DSI <0.8).

4.4.5 Canopy temperature

Canopy temperature ($^{\circ}\text{C}$) was measured at weekly intervals in order to investigate canopy temperature increase during a cycle of drought stress. Data were measured at three dates starting from seven WAS during Trial I and four dates starting from eight WAS during Trial II. Mean data for each measurement date are presented for non-stress and stress treatments for Trial I and II in table 4.5 and 4.6, respectively. All the dates except one (11 WAS during Trial II) showed non-significant genotype x treatment interactions. In the non-stress control, S0, canopy temperature was generally lower for all the genotypes throughout the data measurement period in both trials than in the SFL and S6L treatments. For example, mean canopy temperature over all the genotypes was 21.9, 23.1 and 23.8°C under the S0, SFL and S6L treatments, respectively in Trial I. Similarly, mean canopy temperature in Trial II was 20.6, 22.1 and 21.7°C under S0, SFL and S6L treatments, respectively. The date of measurement for canopy temperature did not have any significant correlation with grain yield, HI or DSI under S0 treatment. However, under stress treatments, significant negative correlations between canopy temperature and each of grain yield and HI and positive correlation with DSI were detected. On the first

Table 4. 5: Mean canopy temperature (°C) for various measurement dates during crop growth in Trial I and their correlations with grain yield, HI and drought susceptibility index (DSI) for eight chickpea genotypes.

Genotypes	7 WAS ¹			8 WAS			10 WAS		
	S0 [¶]	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L
ILC 588	20.6	21.1	22.2	21.4	25.3	25.2	21.7	22.7	23.2
ILC 3182	20.3	19.6	21.9	21.8	23.4	24.7	21.6	24.0	24.5
ICCV 2	19.5	19.9	20.6	22.6	22.4	24.1	23.4	23.7	23.4
Amit	20.9	21.4	22.2	23.5	25.0	23.9	22.8	25.7	23.4
ILC 3279	21.0	22.6	22.0	23.3	24.7	25.5	22.4	23.4	25.1
ILC 10606	21.0	22.0	23.8	23.8	25.3	26.1	23.7	24.0	24.7
ILC 9955	19.9	23.7	23.0	22.8	25.1	26.2	22.6	23.2	24.5
CDC Chico	21.2	20.9	23.4	22.8	23.0	24.8	20.6	22.6	22.4
Mean	20.6	21.4	22.4	22.8	24.3	25.1	22.4	23.7	23.9
Se (+/-)		1.2(1.2) [£]			0.8 (1.4)			0.8(1.1)	
Air Temp (°C)		25.1			25.5			24.9	
Correlations with:									
GY(g plant ⁻¹)	0.24	-0.75*	-0.61	-0.52	-0.71*	-0.59	-0.81	-0.43	-0.63
HI	-0.05	-0.60	-0.41	-0.77	-0.64	-0.53	-0.73	-0.57	-0.75*
DSI	NA	0.13	0.76*	NA	0.33	0.51	NA	0.34	0.48

[¶]S0: Non-stress; SFL: Water withheld from flowering stage; S6L: Water withheld from 6 leaf stage

¹WAS: weeks after sowing

£ Standard error to compare the means within each level of moisture; Values in parenthesis are standard errors to compare means across moisture levels.

Table 4. 6: Mean canopy temperature (°C) for various measurement dates during crop growth in Trial II and their correlations with grain yield, HI and drought susceptibility index (DSI) for eight chickpea genotypes.

	8 WAS ¹			9 WAS			10 WAS			11 WAS		
	S0 [¶]	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L	S0	SFL	S6L
ILC 588	19.6	19.5	20.3	22.1	23.0	23.2	21.4	22.7	22.0	20.8	23.3	23.0
ILC 3182	17.8	18.3	20.6	22.4	23.5	23.1	21.0	22.1	22.5	21.3	22.8	22.8
ICCV 2	17.7	18.9	19.5	22.3	23.2	23.1	21.3	22.5	22.2	22.3	23.2	21.8
Amit	19.3	19.0	20.2	22.7	24.0	22.8	21.3	21.9	21.6	21.0	23.4	22.4
ILC 3279	19.3	20.5	18.5	23.4	23.4	23.1	22.0	22.7	21.8	20.9	23.3	22.8
ILC 10606	18.7	20.1	19.2	22.0	23.9	23.5	20.7	22.5	21.1	20.4	23.1	22.2
ILC 9955	17.9	19.7	20.2	21.9	23.6	22.6	20.7	21.9	21.7	20.2	22.8	22.4
CDC Chico	17.5	19.4	17.9	21.5	23.0	22.4	20.1	22.1	21.0	19.4	22.9	22.3
Mean	18.5	19.4	19.6	22.3	23.4	23.0	21.1	22.3	21.7	20.8	23.1	22.4
Se (+/-)		0.6(0.7) [£]			0.4(0.5)			0.4(0.5)			0.3(0.5)	
Air Temp (°C)		22.7			24.2			23.0			23.1	
Correlations with:												
GY(g plant ⁻¹)	-0.26	-0.66	0.02	-0.33	-0.68	-0.04	-0.13	0.13	0.41	0.16	0.03	-0.07
HI	-0.44	-0.65	0.16	-0.33	-0.68	-0.03	-0.16	0.17	0.47	0.26	0.06	0.23
DSI	NA	0.49	0.10	NA	-0.33	-0.48	NA	0.42	0.17	NA	-0.43	-0.08

[¶]S0: Non-stress; SFL: Water withheld from flowering stage; S6L: Water withheld from 6 leaf stage

¹WAS: weeks after sowing

[£] Standard error to compare the means within each level of moisture; Values in parenthesis are standard errors to compare means across moisture levels.

date of measurement (7 WAS) during Trial I (Table 4.4), the canopy temperature of different genotypes ranged from 19.5 to 21.2 °C under S0, 19.6 to 23.7 °C under SFL and 20.6 to 23.4 °C under S6L treatment. Temperatures increased at later dates of measurement (8 WAS and 10 WAS) from 22.4 to 25.1 °C under S6L.

For the average of all assessment dates in Trial I, ICCV 2 had the lowest canopy temperature (22.0 °C under SFL; 22.7 °C under S6L treatment) while ILC 10606, ILC 9955, ILC 3279 and Amit had the highest canopy temperatures (23.2 to 24.9 °C) under stress treatments. The genotypes with low canopy temperature would be less stressed. Canopy temperature measured at eight WAS during Trial I showed a significant negative association with grain yield and HI but was positively related with DSI (Fig. 4.2). This indicated that cooler canopies were associated with higher grain yield and HI and lower DSI. Genotype ICCV 2 showed lower canopy temperature ($>2^{\circ}\text{C}$) than air temperature followed by ILC 3182, CDC Chico, Amit and ILC 588. On the other hand, ILC 10606, ILC 9955 and ILC 3279 showed canopy temperature equal or greater than air temperature under drought stress treatments and these genotypes would be categorized as stressed.

4.5 Discussion

Grain yield is the most important commercial trait and the ultimate objective in chickpea breeding programs. Grain yield is a complex trait influenced by many environmental and morpho-physiological mechanisms. Selection for grain yield under water stress is often not very effective due to the variability in the stress pattern from place to place and year to year. The efficiency of selection for yield may be increased by indirect selection for morpho-physiological traits having association with yield which can

be measured quickly and easily (Blum *et al.* 1983). The current study reported that stomatal conductance (g_s) decreased from $>275 \text{ mmol m}^{-2} \text{ s}^{-1}$ to $>100 \text{ mmol m}^{-2} \text{ s}^{-1}$ as the plant grew from flowering to maturity under progressive drought stress. Further, the decline in g_s was higher in drought susceptible genotypes as compared to the drought drought tolerant genotypes. This was also evident from the rise in canopy temperature by two degrees Celsius under progressive drought stress. Drought susceptible genotypes showed comparatively higher canopy temperature than tolerant genotypes suggesting that drought susceptible genotypes were not able to maintain adequate transpiration and ultimately transpirational cooling was reduced. Data recorded for g_s and canopy temperature during a cycle of drought showed a strong relationship with grain yield under drought, HI and DSI. This relationship was stronger when g_s and canopy temperature were measured during flowering. Plant stress due to drought can be assessed through the measurement of g_s and canopy temperature. A linear regression of g_s with grain yield under drought, HI and DSI during flowering indicated a reduction in grain yield ($<3.2 \text{ g plant}^{-1}$) and HI (<0.4) at lower g_s ($\leq 250 \text{ mmol m}^{-2} \text{ s}^{-1}$). A severe reduction in grain yield ($<1.3 \text{ g plant}^{-1}$) and HI (<0.2) was noted with further reduction in g_s ($\leq 150 \text{ mmol m}^{-2} \text{ s}^{-1}$). Similar trends were found when g_s were compared to DSI.

The high positive correlation of g_s with grain yield and HI and the negative correlation with DSI indicated the importance of considering g_s for the improvement of grain yield under drought conditions. This g_s -yield relationship agrees with many studies on wheat (Condon *et al.*, 1987; Sayre *et al.*, 1995; Fischer *et al.*, 1998) and barley (González *et al.*, 1999). The current study also found a strong negative relationship of g_s with DSI which emphasizes the role of g_s in the stable performance of genotypes. Blum

et al. (1989) showed a similar relationship in wheat. Hence, use of g_s for crop improvement could lead to higher productivity. One important consideration for using g_s under field conditions is the time required for g_s measurement and the requirement for clear sunny and windless days for measurement. An alternate trait proposed in other crops is canopy temperature.

Canopy temperature showed a significant negative association with grain yield under drought and HI but was positively related with DSI. This relationship indicated that higher canopy temperature accompanied with yield reductions under moisture stress, apparently because plants could not maintain adequate transpiration rates and transpirational cooling was reduced. Similar results have been reported in soybean by McKinney *et al.* (1989) and in wheat by Blum *et al.* (1989). The results from the current study indicated that higher stomatal conductance and cooler canopies were associated with higher grain yield and HI. This association found in Trial I was weaker or absent in Trial II. This lack of agreement with Trial I was most likely due to the difference in time of the year at which stomatal conductance and canopy temperatures were measured in the two trials. The weather outside the greenhouse during Trial II was mainly cloudy and with short winter days which affected overall plant growth. In comparison, during Trial I, the weather outside the greenhouse was mainly sunny with long summer days. Despite these differences, some of the inferences were still obvious. Early flowering and maturity is important in escaping terminal drought stress. Genotypes with early flowering and maturity demonstrated trends to produce higher grain yield and HI under stress. Early flowering and maturity allowed genotypes to escape the severe effects of drought later in

the season. Therefore, earliness is an advantageous trait under conditions of terminal drought stress.

Recently, Berger *et al.* (2006) confirmed the importance of high HI and drought escape through early flowering, podding and maturity of chickpea under natural drought stress environments across India. This finding also agrees with the role of earliness in other crops including wheat and barley under Mediterranean environments (Acevedo *et al.*, 1991; González *et al.*, 1999). Harvest index typically has a strong correlation with grain yield under drought stress as well as non-stress conditions.

The role of canopy temperature as an indirect selection criterion under drought conditions has been documented in wheat (Blum *et al.*, 1982; Pinter *et al.*, 1990; Araghi and Assad, 1998), barley (González *et al.*, 1999) and pearl millet (Singh and Kanemasu, 1983), due to its rapid measurements by hand-held infrared thermometers. Genotypes having lower canopy temperature at mid-day tend to have relatively better water status and are considered to be drought avoidant (Blum *et al.*, 1982; Garrity and O'Toole, 1995).

A strong negative relationship was detected between g_s and canopy temperature under three moisture treatments suggesting that canopy temperature can be a good substitute for stomatal conductance in selecting chickpea genotypes for drought tolerance (Fig. 4.3). The linear regression between stomatal conductance and canopy temperature under non-stress and stress treatments clearly indicated that the traits were significantly associated with each other under non-stress and stress conditions. Although Trial II did not show a significant relationship between grain yield and stomatal conductance or canopy temperature, g_s and canopy temperature did show a strong relationship between

themselves. This indicates that these two traits may be used interchangeably. Time of measurement of g_s and canopy temperature during crop growth is also important. From the data in this study, it is concluded that about one week after the start of flowering is the best time for measurement of stress response for g_s and canopy temperature, and results would be significantly correlated with yield, HI and DSI.

Important considerations for indirect selection for grain yield would be the cost, time and ease of measuring the secondary trait, genotypic correlation with yield, as well as the heritability and genetic variability available. This research recommends the use of stomatal conductance and canopy temperature as rapid indirect selection methods for grain yield under drought conditions. For example, g_s below $250 \text{ mmol m}^{-2}\text{s}^{-1}$ and canopy temperature equal or above air temperature at midday during flowering indicates drought stressed plants and ultimately would cause a yield penalty. With the advent of infrared imaging systems, the possibility of rapid measurement of canopy temperature may increase the efficiency of this trait and reduce the time required for its measurement in the field. These results illustrate the need for further study under field conditions with a wider range of genetic material and to investigate the genetic control of these traits. Identification and use of molecular markers linked with g_s and canopy temperature can speed up the process of selection for drought tolerance in future chickpea breeding programs.

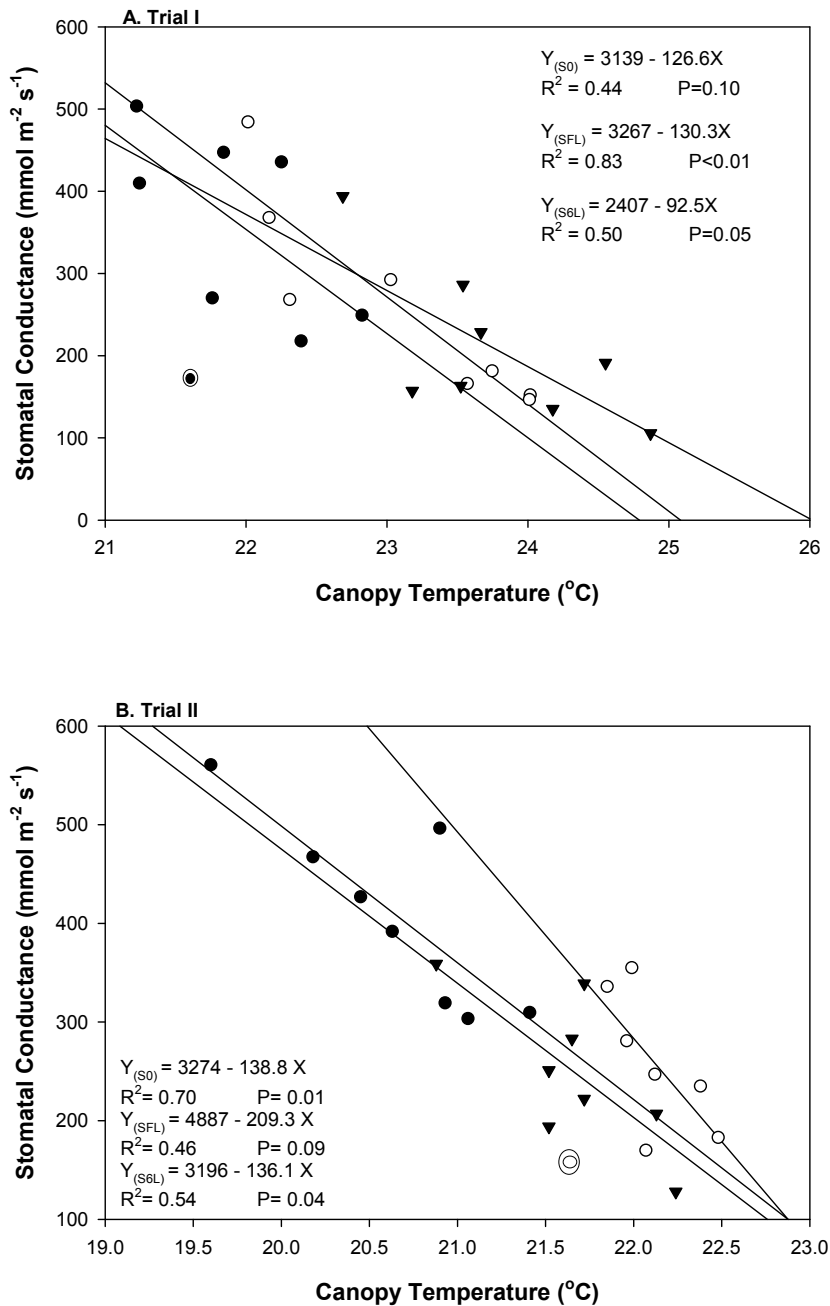


Fig. 4. 3: The relationship of mean stomatal conductance with canopy temperature for eight chickpea genotypes evaluated during Trial I (A) and Trial II (B). Data for stomatal conductance and canopy temperature are the mean of various dates recorded for each genotype under each treatment. Solid circles (●) represent data under S0 treatment, open circles (○) represent data under SFL treatment and (▼) represent data under S6L treatment. The data point circled in graph was excluded before regression analysis.

5. Quantitative trait loci associated with traits determining higher grain yield in chickpea under terminal drought stress

5.1 Summary

Drought is the most important abiotic stress in many chickpea growing regions and occasionally severe drought conditions lead to complete crop failure. The present study was envisaged to characterize a chickpea mapping population of a cross between drought tolerant and susceptible genotypes (ILC 588 and ILC 3279, respectively) developed through single seed descent method. The population consisting of 155 F_{6:7-8} recombinant inbred lines (RILs) was studied under natural drought conditions in the field at Tel Hadya, Syria in 2006 and 2007 and at Breda, Syria in 2007 for various morpho-physiological traits including stomatal conductance (g_s), canopy temperature differential (Tc-Ta) and chlorophyll fluorescence parameters. SSR markers were used to tag quantitative trait loci (QTL) linked to important drought related traits. A genetic linkage map was produced using 52 SSR primers resulting in eight linkage groups. Results obtained from these studies revealed that high harvest index, early flowering, early maturity and high 100-grain weight were the important attributes contributing to higher grain yield under drought. Similarly, results suggested that higher stomatal conductance and cooler canopies can lead to better performance under drought conditions in Mediterranean environments. Analysis of the molecular data revealed 13 genomic regions significantly associated with various traits. Important QTLs detected in this study included five QTLs for harvest index on LG1, LG3, LG4 and LG8 explaining 84% of the

total phenotypic variability. Four QTLs were detected for flowering on LG1, LG3, LG4 and LG6 and four for maturity on LG1, LG3 and LG7. One QTL was detected for stomatal conductance on LG7 explaining 9% of total variability, and three QTLs for canopy temperature differential on LG1, LG3 and LG6 explaining 39% of total phenotypic variability.

5.2 Introduction

Chickpea (*Cicer arietinum* L.) is an important legume crop in the Semi-Arid Tropics (SAT) and the West Asia and North Africa (WANA) regions, and is becoming an important legume crop in new regions like Australia and North America. Although chickpea is known for its better drought tolerance than most other cool-season legumes, drought does reduce yields and can even lead to total crop failure. In both Mediterranean and sub-tropical climates, seed filling in chickpea is subject to terminal drought, which limits seed yield (Turner *et al.*, 2001). Many physiological processes associated with crop growth and development, are influenced by water deficits (Hsiao, 1973; Boyer and McPherson, 1975; Begg and Turner, 1976; and Turner and Begg, 1978).

Early maturing chickpea varieties that escape terminal drought have been developed (Kumar and Abbo, 2001), but early maturity places a ceiling on the potential yield and limits the crop's ability to exploit extended growing periods. Increasing the drought avoidance of the crop should help to stabilize yields at higher levels than possible with escape (Johansen *et al.*, 1997).

In chickpea, the focus of drought resistance is on the ability to sustain greater biomass production and crop yield under a seasonally increasing water deficit, rather than

the physiological aptitude for plant survival under extreme drought shock (Serraj and Sinclair, 2002). This has led to the focus on escape and avoidance strategies such as early maturity (Kumar and Abbo, 2001) and large root systems (Saxena *et al.*, 1995; Singh *et al.*, 1995).

Several physiological criteria for selecting resistant genotypes have been proposed and demonstrated in other crops. For most plants, drought avoidance is achieved primarily through regulation of stomatal conductance in response to soil and atmospheric water deficit (Cohen, 1970; Cowan, 1982; Schulze, 1986; Dawson and Ehleringer, 1993; Meinzer, 1993). Stomatal closure can serve as a rapid and effective drought avoidance response, however, prolonged stomatal closure is not sustainable as stomatal CO₂ uptake is also reduced and ultimately limits photosynthetic assimilation and growth (Farquhar and Sharkey, 1982; Schulze *et al.*, 1987). Ritchie *et al.* (1990) observed that the most drought resistant wheat genotypes had greater stomatal conductance (g_s) under water-stress conditions than susceptible genotypes. Wheat yield was improved under drought conditions by higher stomatal conductance (Fischer *et al.*, 1998). González *et al.* (1999) also found a strong association between barley yield under drought and higher stomatal conductance. An important consequence of stomatal closure that occurs when plants are subject to water stress is that energy dissipation is decreased so leaf temperature tends to rise. Since a major role of transpiration is leaf cooling, canopy temperature and its reduction relative to ambient temperature is an indication of the role of transpiration in cooling the leaves. Thus, interest is increasing in using canopy temperature when breeding for drought tolerance. This involves selection of genotypes that maintain lower canopy temperature as compared with other genotypes under the

same field conditions. Relatively lower canopy temperature in drought stressed crop plants indicates a better capacity of taking up soil moisture and for maintaining a better plant water status. Canopy temperature depression was an effective technique for screening wheat (Blum *et al.*, 1982; Pinter *et al.*, 1990) and pearl millet (Singh and Kanemasu, 1983) genotypes for resistance to drought.

Photosystem II (PSII) is an important component of plant photosynthesis, and is particularly sensitive to water deficit (Lu and Zhang, 1999). The potential of using chlorophyll fluorescence assessment in screening drought resistant plants has been reported (Maxwell and Johnson, 2000; Baker and Rosenqvist, 2004). Chlorophyll fluorescence is widely accepted as an indication of the energetic behavior of the photosynthetic system. Several fluorescence parameters such as initial fluorescence (F_o , the fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidized), maximal fluorescence (F_m , the fluorescence level when Qa is transiently fully reduced), variable fluorescence (F_v , $F_m - F_o$) and maximum quantum efficiency of PSII (F_v/F_m), have been widely used for such studies in various species under diverse growth conditions (Araus *et al.*, 1998; Fracheboud *et al.*, 2004). Parameters, such as F_o and F_m measured during the grain filling stage of wheat under drought stress showed high genetic correlation with grain yield (Araus *et al.*, 1998), suggesting that these parameters can be used as indicators to evaluate the yield performance across genotypes under water deficit conditions (Araus and Hogan, 1994). However, little is known about the possibility of screening large populations under field drought conditions using these parameters. A better understanding of the genetic basis of these parameters and their

association with drought tolerance could contribute to their use in breeding strategies for dry environments.

Breeding for drought tolerance is generally considered slow due to the quantitative and temporal variability of available moisture across years, the low genotypic variance in yield under these conditions and inherent methodological difficulties in evaluating component traits (Ludlow and Muchow, 1990), together with the highly complex genetic basis of this trait (Turner *et al.*, 2001). The availability of genetically fixed Recombinant Inbred Line (RIL) populations combined with DNA markers and rigorous phenotyping should improve the ability to study and manipulate drought resistance traits (Crouch and Serraj, 2002). As drought tolerance is a complex trait and screening is laborious, there is a need to exploit molecular techniques. Recent studies suggested that highly homozygous recombinant inbred lines can be used for drought tolerance studies (Abbo *et al.*, 2002) and development of molecular markers can be helpful to improve yield stability under drought stress.

In this research, homozygous F_{6:7-8} recombinant inbred lines (RILs) developed at ICARDA, Syria were used to study various drought tolerance indicator parameters and simple sequence repeat (SSR) markers were used for linkage analysis. Hence, the following specific objectives were addressed: a) to measure the phenotypic expression of agronomic and physiological traits associated with drought tolerance in a set of RILs from an intraspecific chickpea population, b) to genotype the RILs with SSR markers to develop a genetic linkage map, and c) to study the genetic control of drought tolerance by QTL analysis.

5.3 Materials and Methods

5.3.1 Plant material

An intraspecific cross of kabuli chickpea between cultivar ILC 588 and a landrace ILC 3279 was used to develop a RIL population. ILC 588 is drought tolerant and ILC 3279 is drought susceptible based on their yield performance under drought conditions (personal communication, R.S. Malhotra, ICARDA, Syria). The cross was advanced through single seed descent method in the field and plastic house at International Center for Agricultural Research in the Dry Areas (ICARDA), Syria. A total of 155 F_{6,7-8} RILs along with parental genotypes were evaluated for yield components and drought tolerance during 2006 and 2007 at two locations i.e. Tel Hadya and Breda, Syria.

5.3.2 Site description

The study was conducted under field conditions in three environments i.e. one location (Tel Hadya, Syria) during 2006 and two locations (Tel Hadya and Breda, Syria) during 2007. The 25 year average annual rainfall at Tel Hadya is 350 mm while at Breda is 250 mm. The soil characteristics at the three experiments are summarized in Table-5.1. Soil analysis was carried out at Soil, Plant and Water Analysis Laboratory, ICARDA, Syria using the hydrometer method and USDA textural classification for soil textural classes. Soil pH was determined in 1:1 soil – water ratio (Jackson, 1958), EC in 1:1 soil – water ratio extract, organic matter (OM) by the Walkley and Black method (Hesse, 1971), total nitrogen (N) by the Kjeldahl method (Hesse, 1971), available phosphorus (P) by Olsen's method (Olsen *et al.*, 1954) and extractable potassium (K) by using an ammonium acetate extractant and flame photometer. Field capacity and wilting point of

the soil were determined by pressure plate extractor (1 and 15 bar). Soil particle size analysis was conducted by the hydrometer method (bouyoucos hydrometer).

5.3.3 Experimental procedure

Each trial consisted of two replicates in an alpha-lattice design. Seeds were sown in one meter row plots with a ten centimeter plant to plant and 45 cm row to row distance. Due to a shortage of seed, some RILs were not included in the trials during 2007 while some new lines were included whose seed was increased during the previous year. The total number of RILs tested in the first trial at Tel Hadya during 2006 (TH06) was 155 while 142 RILs were tested during 2007 at Tel Hadya site (TH07) and 108 at Breda site (BR07). Parental genotypes were included in all trials. Trail TH06 was planted on March 22 and harvested on June 28, TH07 was planted at Tel Hadya on March 11 and harvested on June 18, and BR07 was planted on January 4 and harvested on June 09. Seeds were treated with the fungicides carboxin, thiram, attapulgate clay at the rate of 3 g/kg for the control of *Rhizoctonia solani*, *Helminthosporium*, *Fusarium* and *Pythium* species. The fungicide chlorothalonil was sprayed at the rate of two liters per hectare (720 g/L of chlorothalonil) during the vegetative period for the control of *Aschochyta*.

5.3.4 Measurements for various traits

Measurement of yield and yield related traits

Grain yield under drought and associated yield components are important in determining the performance of genotypes under drought. The five middle plants from each row were harvested individually for subsequent measurements. All the

measurements were made on an individual plant basis and the means of five plants were used for analysis for yield and related traits. At maturity, plants were harvested by cutting at ground level and placing each plant in separate bags. Plants were oven-dried at 45°C for 48 hours before weighing. Traits measured were grain yield (g plant^{-1}), above ground biomass (g plant^{-1}), number of grains per plant, number of pods per plant, 100-grain weight (g plant^{-1}). Harvest index (HI) was calculated according to the formula:

$$\text{HI} = \text{Grain weight} / \text{Total above ground dry weight}$$

Measurement of morphological traits

Days to flowering, days to maturity, reproductive period and plant height are important morphological traits to characterize genotypes under drought conditions. Data were collected on days from sowing to flowering by calculating the difference of days from date of sowing to the date when 50% of the plants in a line showed the first fully open flower. Days from sowing to physiological maturity were recorded by calculating the difference of days from date of sowing to the date when 90% of the plants had turned colour. The reproductive growth period was calculated as the days between the start of flowering and physiological maturity. Plant height (cm) was measured just prior to physiological maturity by taking four readings on each line excluding border plants and averaging before analysis.

Measurement of drought tolerance score (DTS)

Drought tolerance score (DTS) (Singh *et al.*, 1997) is a quick visual assessment of genotypes for their performance under drought. The rating was done visually at the late pod-filling stage, when the crop was approaching maturity. A scale from 1 to 9 was used

with 1 being drought stress free and 9 being inability to set seed in the prevailing stress conditions. The description of 1-9 scale is as follows:

1 = free, no visible symptoms of damage, early flowering, profuse podding and seed formation, normal maturity, high productivity;

2 = highly resistant, early flowering, profuse podding and seed formation, normal maturity, high productivity;

3 = resistant, early flowering, normal podding and seed formation, normal maturity, relatively high productivity;

4 = moderately resistant, early-medium flowering, normal podding and seed formation, normal maturity and relatively high productivity;

5 = intermediate, early-medium flowering, normal podding but many without seeds, normal maturity and moderate productivity;

6 = moderately susceptible, early-medium flowering, few podding and many without seeds, forced maturity and moderate productivity;

7 = susceptible, late flowering, few pods and many without seeds, forced maturity and low productivity;

8 = highly susceptible, late flowering, pods rare and mostly without seeds, forced maturity and nominal productivity;

9 = all plants dried without any seed, late flowering and no pod formation or productivity.

Measurement of stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$)

For most plants, drought avoidance is achieved primarily through regulation of stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) in response to soil and atmospheric water deficit (Cohen, 1970; Meinzer, 1993). However, prolonged stomatal closure is not sustainable as stomatal CO_2 uptake is also reduced and ultimately limits photosynthetic assimilation and growth (Schulze et al., 1987). Higher stomatal conductance was associated with higher yields under drought in various crops (see chapter 2). Stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) was measured with a steady state porometer (Li-1600, LI-COR Inc., Lincoln, NE), with cuvette conditions set to ambient. The Li-Cor 1600 operates on the null balance principal. When the transpiring intact leaf is inserted into the cuvette (Fig. 2.4, chapter 2), it raises the humidity level inside the cuvette. To balance this increased humidity, an internal flow controller increases the flow of dry air to the cuvette until it balances the humidity to a predetermined set point which is ambient RH. As different genotypes transpire at different rates, different amounts of dry air are required to reach the set point. This difference is used to compute the stomatal conductance. All the measurements were recorded on the third from top fully expanded well-lit leaf around mid day (10.00 to 14.00) at three times in TH06 at weekly intervals and one time in TH07. The first measurement during 2006 was carried out a week before the start of flowering. Measurement during 2007 was carried out during the second week after the start of flowering. All the measurements were made on clear, sunny and calm days.

Measurement of canopy temperature (°C)

Relatively lower canopy temperature in drought stressed crop plants indicates a better capacity for taking up soil moisture and for maintaining better plant water status. Canopy temperature was effective in screening different crops for resistance to drought (see Literature Review for details). Measurements of canopy temperature (T_c) were carried out at the same time or within one day of the measurement of g_s . A hand-held infrared thermometer (Everest Interscience Inc., Fullerton, CA) with 4° field of view, was used to measure canopy temperature (°C). The data for each plot were the mean of four readings, each of which was the average of 10 readings, taken from both sides of each row to remove the effect due to the direction of the sun, at an angle of approximately 30° to the horizontal, in a range of directions such that it covered the plant canopy. All the measurements were made on clear, sunny and calm days. Air temperature (T_a) and relative humidity were recorded with an automatic weather station located within 100 meters of the trial site. T_c-T_a was computed and analyzed.

Measurement of chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters are considered as traits associated with drought tolerance in crop plants. These parameters can be used as indicators to evaluate the yield performance across genotypes under water deficit conditions (Araus and Hogan, 1994). Chlorophyll fluorescence parameters were measured using Handy PEA (Hansatech Instruments, Norfolk, UK) following the manufacturer's instructions. Dark adaptation period for all the measurements was about 25 minutes. Clips (Fig. 5.1) were used to provide 25 minute dark adaptation to the leaves. Two readings were taken on the

third fully expanded leaf from the top for each RIL and parental lines and averaged before analysis. Data were recorded three times in TH06 at weekly intervals starting one week prior to the start of flowering, two times in TH07 at weekly intervals during flowering and one time in BR07 during the flowering period. The following parameters were recorded.

F_o = initial fluorescence level when plastoquinone electron acceptor pool (Q_a) is fully oxidized.

F_m = fluorescence level when Q_a is transiently fully reduced.

F_v = variable fluorescence (F_m-F_o).

F_v/F_m = Maximum quantum efficiency of photosystem II (PSII).

5.3.5 Measurement of soil moisture

Soil moisture availability throughout the growing season is crucial for crop growth and development. Through the knowledge of soil moisture status of a particular environment, crop phenology can be adjusted to avoid drought stress in some environments. Soil moisture was monitored by neutron probes (Didcot Instrument Co. LTD., Abingdon, Oxon, England) at 9 points scattered throughout the trial at Tel Hadya during both years. Measurements were taken up to 180 cm depth. The instrument was calibrated before the start of the measurements. Tubes were installed and measurements were carried out at biweekly intervals starting from sowing until harvesting of the experiment. The neutron moisture probes consists of a probe containing a source of fast neutrons that move radially outward from the source, a thermal neutron detector, and the associated electronic equipment to supply power for the detector and display the results

(Fig. 5.2A & B). The radioactive source in this instrument emits fast neutrons. When the fast neutrons encounter hydrogen in the soil, they lose energy and are slowed down. Most of the hydrogen in the soil is associated with soil water. The neutrons, having no electric charge, cannot be detected directly. Therefore, a gas is used (usually, boron trifluoride or helium-3) which can cause neutron absorption. The slow neutrons enter the nucleus of the gas, the nucleus is raised to a high energy state and photons are emitted from the nucleus. The electronic counting device of neutron probes measures the number of thermalized neutrons which is proportional to the soil water content. Soil samples were also analyzed and bulk density was determined to convert neutron probe measurements into moisture content. Due to safety reasons, neutron probes were used to measure soil moisture from 15 cm to 180 cm depth. In the upper 0-15 cm section of soil, water contents were measured gravimetrically. For this purpose, fresh soil samples were weighed and subsequently oven-dried at 100°C for 48 hours to obtain dried weight.

5.3.6 Statistical analysis

Individual trial: Each trial was analyzed using the following linear additive mixed effects model

$$Y_{ijk} = \mu + r_i + b_{ij} + g_k + e_{ijk}$$

where Y_{ijk} is the observation of a trait recorded on an experimental plot under the genotype k in incomplete block j of replicate i , μ is the general mean, r_i the effect of replicate i , b_{ij} the effect of block j within replicate i , g_k the effect of genotype k , and e_{ijk} the experimental error from the plot. The effects of replications and blocks within



Fig. 5. 1: Chlorophyll fluorescence was measured using (A) Handy PEA (Hansatech Instruments, Norfolk, UK). Clips (B) were fixed on leaves 25 minutes before the measurement for dark adaptation.

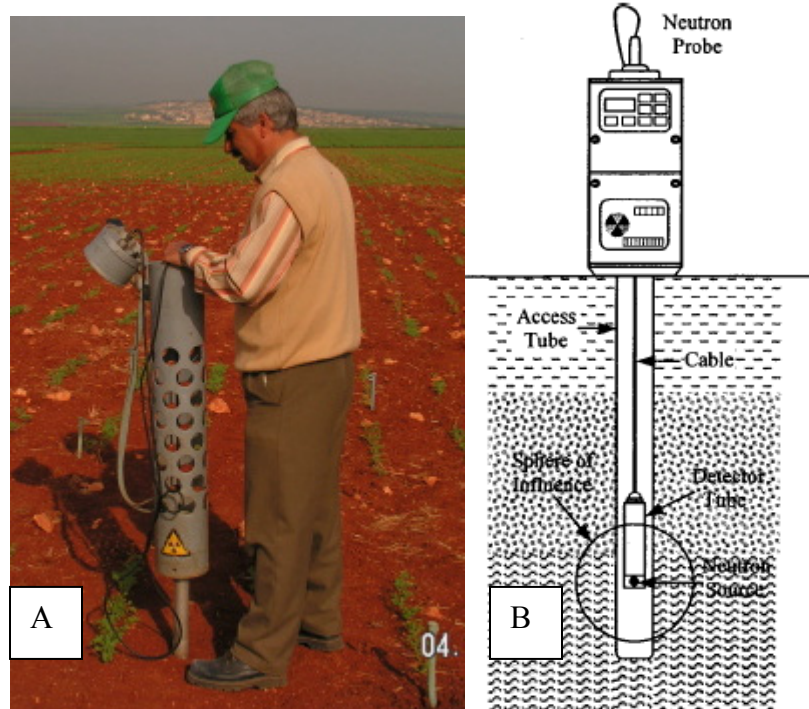


Fig. 5. 2: Monitoring of soil moisture using neutron probes (A). Nine access tubes up to 180 cm soil depth were installed throughout the experiment site. Photo was taken during 2006 at experiment site Tel Hadya, Syria. Schematic diagram of neutron probe (B) showing parts under the ground (source of diagram (B): Li *et al.*, 2003).

replications were assumed independent and normally distributed random variables with means zero. The experimental errors (i.e. the plot effects) e_{ijk} were assumed independent and normally distributed with mean zero and variance σ_e^2 , and independent of the other random factors in the model. The variance of genotype effects, g_k , was denoted by σ_g^2 .

A measure of broad-sense heritability of trait Y is the ratio of genetic variance (σ_g^2) to phenotypic variance ($\sigma_g^2 + \sigma_e^2$).

$$h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$$

Multi-environment trials: The data from the multi-environment trials were combined and the above model was modified to incorporate fixed effects due to environments while genotype x environment interaction effects were assumed random with mean zero and variance σ_{ge}^2 . The experimental error variances σ_e^2 were assumed constant over the trials. The heritability of a given trait, on a means basis, from all the trials was estimated as follows:

$$h^2 = \sigma_g^2 / \{ \sigma_g^2 + \sigma_{ge}^2 / L + \sigma_e^2 / (RL) \}$$

where R is the number of replications in each trial and L is the number of locations or environments.

Estimation of the variance components in the above model, and its generalized version for multi-environments, were carried out using residual maximum likelihood (REML) method provided in the PROC MIXED procedure of SAS 8.02 (SAS Institute, Cary, N.C., USA, 1999). Standard error for heritability was estimated as explained by Singh and Ceccarelli (1995).

5.3.7 Genotyping

Fresh leaves from 4 to 6 week old seedlings were collected at TH06 for DNA isolation from parents and each of the RILs. Genomic DNA was extracted following the CTAB protocol described by Saghai-Marroof *et al.* (1984). Leaf tissues were collected in the field and kept in liquid nitrogen until stored in freeze dryers. After DNA extraction, the DNA was quantified using a spectrophotometer and SoftmaxTM Pro software package. After DNA quantification, the tubes containing DNA were labeled and stored at -20°C as stocks. Diluted DNA was used for PCR reactions at a concentration of 50 ng/μl.

Genetic mapping was carried out using SSR markers. Parental lines were screened with 260 SSR primer pairs obtained from Hüttel *et al.* (1999), Winter *et al.* (1999), Sethy *et al.* (2003) and Lichtenzveig *et al.* (2005). Markers which were polymorphic on the parents were selected for screening on the population. Amplification was done using a MJ Research PTC-100 or PTC-200 programmable thermal cycler. The program used for primers starting with TA-, TR-, TS- and H- included three minutes of initial denaturation at 94 °C followed by 30 cycles at 94 °C for one minute, a 50 second annealing step at 50 °C and a one minute elongation step at 60 °C. A final extension step at 60 °C for five minutes was applied. The program used for primers starting with NCPGR- included two minutes of initial denaturation at 94 °C followed by 34 cycles at 94 °C for 20 second, a 50 second annealing step at 50 °C and a 50 second elongation step at 72 °C. A final extension step at 72 °C for seven minutes was applied. The program used for primers starting with GA- and CASTMS- included three minutes of initial denaturation at 94 °C followed by 29 cycles at 94 °C for one minute, a 50 second annealing at 55 °C and one minute elongation at 72 °C. A final extension step at 72 °C for eight minutes was applied.

PCR products were separated on 6% polyacrylamide gels and stained with silver nitrate. Bands were scored for individual lines. To accommodate RILs and parents in two 66-well gels, 128 RILs were short listed out of 155 RILs on the basis of their performance for DTS in TH06. RILs within half standard deviation unit around mean were excluded from genotyping due to the fact that RILs with contrasting response for the trait contribute more for QTL identification.

Linkage and QTL analysis

Chi-square analysis ($P < 0.05$) was applied to test the segregation of the mapped markers against the expected Mendelian segregation ratio of 1:1 for RILs. Genetic linkage groups of the SSR markers were determined using the GROUP command of MAPMAKER/EXP program version 3.0 (Lander *et al.* 1987) at a LOD score of 3.0. The order of the markers within a linkage group was determined using the COMPARE command at a LOD score of 3.0. Additional markers were added using the TRY command at a LOD threshold of 3.0. The best order of the markers was then verified using the RIPPLE command with a LOD score of 3.0. The QTL location and effect were estimated by composite interval mapping (CIM) using the Windows QTL Cartographer program version 2.5 (Wang *et al.*, 2005). The CIM performs first a multiple regression involving all the markers, then uses the markers explaining most of the genetic variation as co-factors when performing the classical single interval mapping. The standard model of Zmapqtl procedure (Basten *et al.* 1994) was used in the analysis by scanning the genome every 2 cM. The threshold levels to declare significant QTL were determined by performing 1000 permutations of the data by maintaining the chromosome-wise type I

error rate of 0.05 (Churchill and Doerge, 1994). The additive effects and R^2 (% explanation for variability) of the detected QTL were estimated by the Zmapqtl procedure.

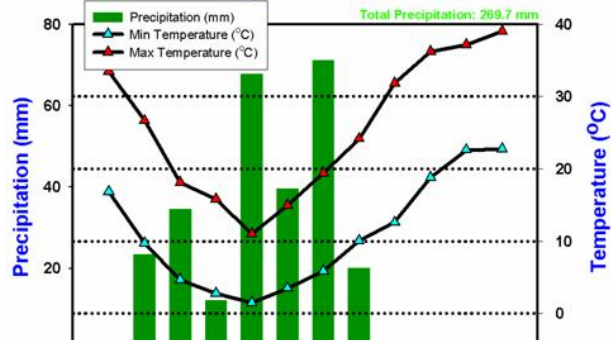
5.4 Results

5.4.1 Weather

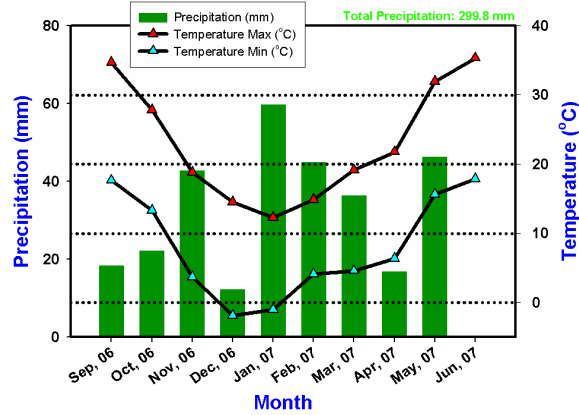
Environmental data regarding precipitation, minimum and maximum temperatures are shown in Fig. 5.3 for the three experimental sites viz. TH06, TH07, and BR07. Total precipitation received at TH06 (from September 2005 to August 2006) was 270 mm, at TH07 for the same period was 300 mm, and at BR07 for the same period was 267 mm. Most of the crop growth period during 2006 was drier as compared to 2007 at Tel Hadya where the crop received intermittent rainfall with high temperature during later growth stages in May. Total precipitation received during the trial period at TH06 was only 25.7 mm and at TH07 Location was 72.6 mm while at BR07 received about 191 mm during the trial period.

Mean monthly minimum temperature at TH06 ranged from 5°C to 12 °C from March to May and further increased to 20 °C during June. Similarly, mean monthly maximum temperature ranged from 20 °C to over 30 °C from March to May and further increased to 35 °C during June. Mean minimum temperature during April 2007 at Tel Hadya was lower in March as compared to 2006 at the same time but abruptly increased during May to 30 °C. Precipitation received during May 2007 was over 40 mm. Similarly, minimum temperature at Breda ranged from below 0 °C in January to over 5 °C in April

**Monthly Precipitation and Ave Min & Max Temp (°C)
at Tel Hadya, Syria
Sep, 2005 to Aug, 2006.**

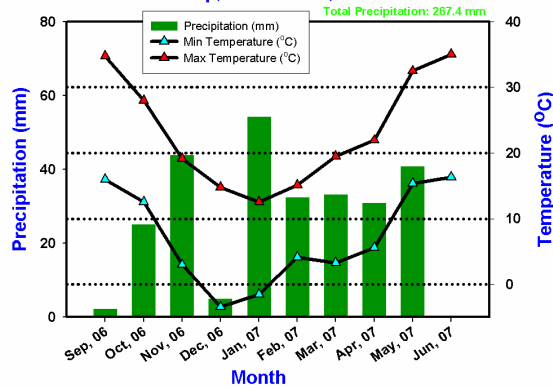


**Monthly Precipitation and Ave Min & Max Temp (°C)
at Tel Hadya, Syria
Sep, 2006 - June, 2007**



Source: ICARDA Meteorological Database - Daily Data

**Monthly Precipitation and Min and Max Temp (°C)
at Breda, Syria
Sep, 2006 - June, 2007**



Source: ICARDA Meteorological Database - Daily Data

Fig. 5. 3: Monthly precipitation and mean minimum and maximum temperature (°C) during growing season at (a) Tel Hadya, Syria during 2006, (b) Tel Hadya, Syria during 2007 and (c) Breda, Syria during 2007.

and increased to 15 °C in May. Mean maximum temperature ranged from 12 °C in January to 22 °C in April and 33 °C in May, 2007.

5.4.2 Site description

The soil at the TH06 trial site contained 64% clay, 28% silt and 8% sand (Table 5.1). The soil at the TH07 trial site contained a similar ratio with 62% clay, 25% silt and 13% sand. The soil at the BR07 site contained 40% clay, 29% silt and 30% sand. Organic matter at the three sites was similar around 1% with pH around 8.5 and EC 0.2 mS cm⁻¹. The three sites were relatively rich in total nitrogen (Table 5.1). Soil water contents were monitored by neutron probes up to 180 cm depth during the growing season at TH06 and TH07 (Fig. 5.4 & 5.5).

Changes in soil moisture content from sowing to harvesting were observed down to 75 cm depth. Moisture level from 75 cm to 180 cm remained relatively unchanged during the entire growing season. During 2007, there was intermittent rainfall, hence frequent changes in the soil moisture content in the upper layers can be seen in Fig. 5.5. In contrast, relatively normal rainfall patterns occurred in 2006 resulting in increased soil dryness from sowing towards harvesting with depleting moisture contents in upper layers.

5.4.3 Yield and yield components

A comparison of mean grain yield (g plant⁻¹) over all the genotypes at the three environments is given in Fig. 5.6. Mean grain yield was higher at Tel Hadya as compared to Breda. At Tel Hadya, grain yield was higher during 2007 than 2006. Fig. 5.7 shows the frequency distribution of the RILs for grain yield per plant for the three environments.

Table 5. 1: Soil analysis for the three trials for drought tolerance in chickpea.

Location	pH (1:1)	E.C.*	O. M.	Total N	Olsen P	Extr. K	F. C.	Wilting Point	Available Water	Mechanical Analysis		
		(1:1)								Clay	Silt	Sand
		mS/cm	%	ppm	ppm	ppm	%	%	%			
Tel Hadya, 2006	8.5	0.2	1.1	681	2.6	358	39.6	27.2	12.3	63.8	27.8	8.4
Tel Hadya, 2007	8.4	0.2	1.0	669	6.6	414	39.0	25.2	13.7	61.7	25.1	13.2
Breda, 2007	8.8	0.2	1.2	729	6.1	166	34.2	18.8	15.4	40.6	29.4	30.0

*EC: electrical conductivity, OM: organic matter, Total N: total nitrogen, Olsen P: Olsen phosphorous, Extr. K: Extractable potash, F.C.: field capacity

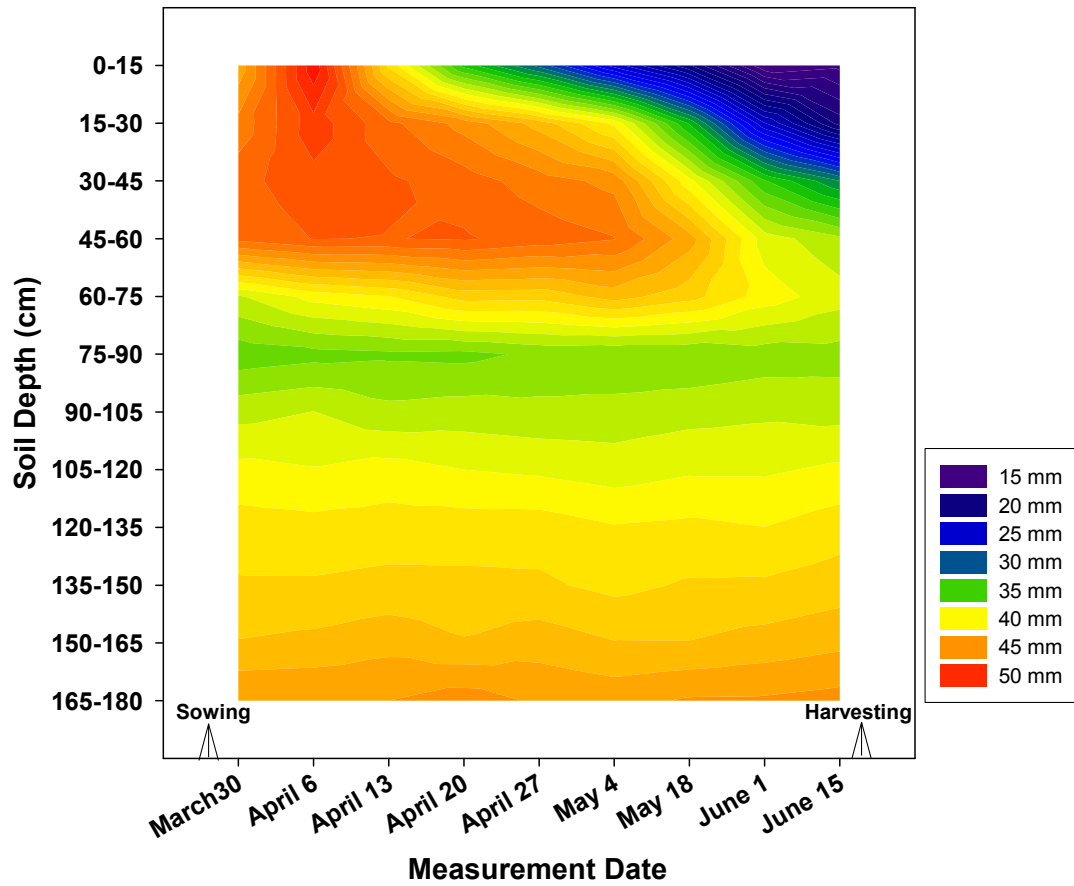


Fig. 5. 4: Graph showing soil moisture content (mm) at various depths measured using neutron probes in the field trial at Tel Hadya, Syria during 2006

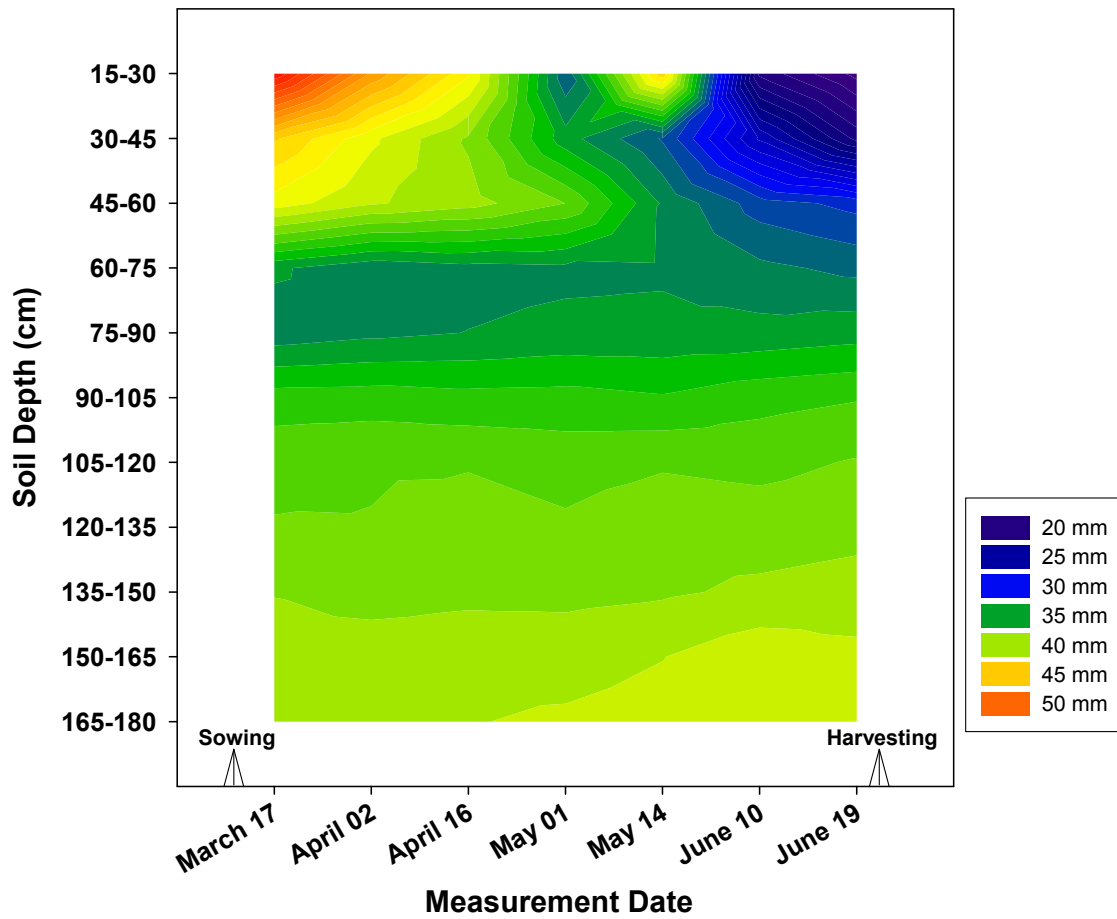


Fig. 5. 5: Graph showing soil moisture content (mm) at various depths measured using neutron probes in the field trial at Tel Hadya, Syria during 2007

The drought susceptible parent ILC 3279 had lower mean grain yield than the population mean, while the drought tolerant parent ILC 588 had higher mean grain yield than the population mean in all three experiments. The means, minimum and maximum, skewness, kurtosis along with standard deviation for parental genotypes and RILs for yield and yield components is provided in Table 5.2. The parents differed significantly for grain yield plant^{-1} under drought and for yield components in all three trials. The difference among RILs was also significant for grain yield and yield components.

The minimum grain yield per plant obtained during TH06 was $0.09 \text{ g plant}^{-1}$, while the maximum grain yield was obtained during TH07 ($6.88 \text{ g plant}^{-1}$). Regarding shoot dry weight, the difference between the two parents was significant during TH06 but not during TH07 and BR07. The minimum 100-grain weight for the RILs (13 g) was obtained during BR07, while the maximum (33 g) occurred during TH06. The number of grains per plant ranged from 0.3 during TH06 to 27 during TH07. Similarly, the number of pods per plant ranged from 0.7 during TH06 to 27 during TH07. In general, only a single seed per pod was obtained in this population.

5.4.4 Harvest index

The means, minimum and maximum along with standard deviation for parental genotypes and RILs for harvest index is provided in Table 5.3. The frequency distribution for harvest index in the three trials is presented in Fig. 5.8. The parents differed significantly for harvest index in all the three trials. The range observed in TH06 trial was greater as compared to the other trials. In general, the harvest indices at BR07 trial were low. The minimum harvest index obtained was 0.02 and the maximum harvest index was 0.59 obtained during TH06.

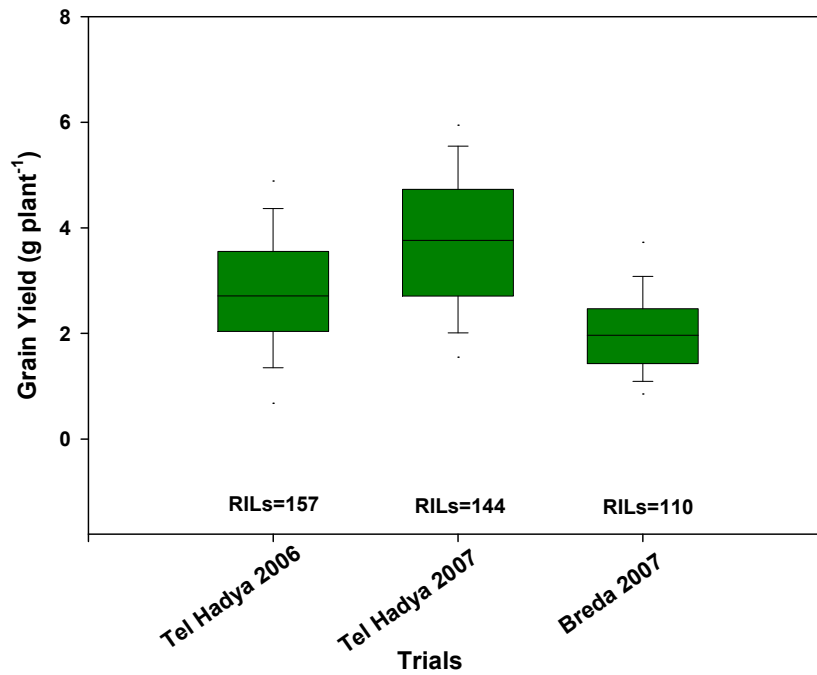


Fig. 5. 6: Box and whisker plot showing mean and variance of RILs of a cross of ILC 588 x ILC 3279 for grain yield (g plant⁻¹) at Tel Hadya, Syria during 2006 and 2007 and Breda, Syria during 2007.

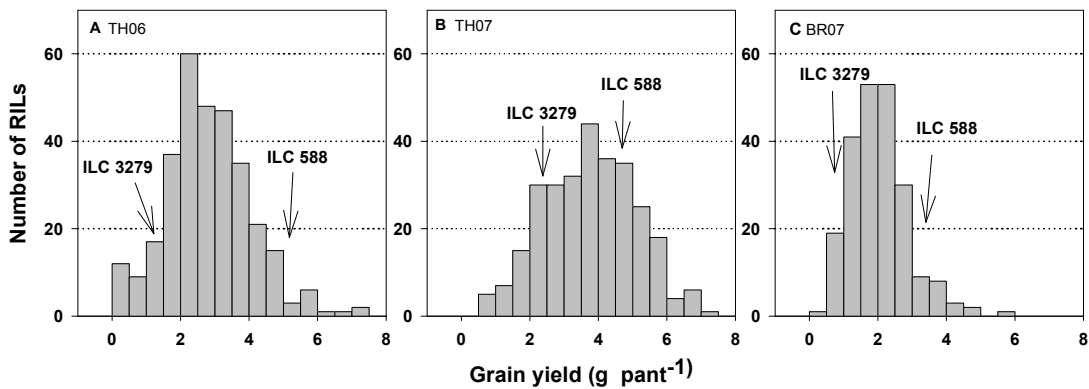


Fig. 5. 7: Frequency distribution of RILs of a cross of ILC 588 x ILC 3279 for grain yield (g plant⁻¹) for trials conducted at Tel Hadya, Syria during 2006 and 2007 and at Breda, Syria during 2007. Arrows shows the position of both parents of the population in the distribution.

Table 5. 2: Means, standard deviation and range of the RILs of the cross ILC 588 x ILC 3279 and means for parental genotypes for various agronomic traits under field drought stress conditions during 2006 and 2007.

Trait ^a	Trial ^b	#	Parents		RILs						
			RILs	ILC	ILC	Min ^c	Max	Mean	Se	Skew	Kurt
				588	3279						
GY	TH06	157	5.02	0.16	0.09	5.96	2.82	0.63	0.1	0.5	
	TH07	144	4.05	2.67	0.77	6.88	3.76	0.72	-0.1	-0.2	
	BR07	110	2.53	0.86	0.55	4.15	2.05	0.50	0.4	0.2	
SDW	TH06	157	10.10	3.39	3.19	14.50	6.99	1.29	0.9	1.4	
	TH07	144	8.30	7.81	4.72	14.35	9.20	1.80	0.2	0.4	
	BR07	110	10.49	9.05	5.58	18.20	10.53	1.70	0.9	1.4	
100-GW	TH06	157	32.5	21.6	14.7	32.5	23.5	2.3	0.1	2.2	
	TH07	144	25.1	23.1	16.6	28.6	22.7	2.1	-0.1	-0.4	
	BR07	110	26.3	20.0	13.0	26.5	20.7	1.6	-0.3	-0.1	
Grain P ⁻¹	TH06	157	15.8	0.9	0.3	26.5	12.0	2.6	0.0	0.5	
	TH07	144	16.2	11.6	6.1	27.4	16.7	4.0	0.1	0.1	
	BR07	110	9.6	4.3	2.8	17.2	9.7	2.8	0.2	0.3	
Pods P ⁻¹	TH06	157	17.4	0.7	0.7	23.2	11.8	2.5	-0.2	0.1	
	TH07	144	16.6	11.4	5.9	26.9	17.1	4.0	0.0	-0.0	
	BR07	110	11.4	7.4	5.2	21.1	12.1	2.2	0.3	0.7	
Grain Pod ⁻¹	TH06	157	0.9	1.2	0.2	1.4	1.0	0.2	-2.1	19.5	
	TH07	144	1.0	1.0	0.8	1.2	0.9	0.1	0.0	0.8	
	BR07	110	0.8	0.6	0.4	1.1	0.8	0.07	-0.3	0.7	

^a GY, Grain yield (g plant⁻¹); SDW, shoot dry weight (g plant⁻¹); 100-GW, hundred grain weight (g); Grain P⁻¹, number of grains per plant; Pods P⁻¹, number of pods per plant; Grain Pod⁻¹, number of grains per pod

^b TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

^c Min, minimum; Max, maximum; se(+/-), standard error (+/-); Skew, skewness; Kurt, kurtosis

5.4.5 Drought tolerance score (DTS)

The means, minimum and maximum along with standard deviation for parental genotypes and RILs for drought tolerance score is provided in Table 5.3. The frequency distribution for drought tolerance score in all the three trials is presented in Fig. 5.9. The parents differed significantly for DTS in all three trials. The range observed in TH06 trial was greater as compared to the other trials. The DTS for ILC 588 was better at TH06 as compared to the other two trials and DTS for ILC 3279 was poorest at TH06 as compared to the other two trials. Minimum DTS was 2 and maximum was 9 during the TH06 trial.

5.4.6 Phenology

Mean values and other relevant statistical parameters for traits including number of days from sowing to flowering (DFF), days to physiological maturity (DM), reproductive period (RP) and final plant height (cm) are presented in Table 5.4. The frequency distribution for days to flowering and maturity are presented in Fig. 5.10 and 5.11. The parents differed significantly in DFF, DM, RP and plant height in all three trials. ILC 588 flowered and matured early as compared to ILC 3279. Reproductive period was longer for ILC 3279 as compared to ILC 588, with the exception of trial TH07 where an abrupt switch to high temperatures during May and June forced plants to mature early. The duration of the trial at BR07 was longer than TH06 and TH07 because of much earlier sowing associated with slow early season crop growth due to cold weather. The earliest RIL flowered in 42 days at TH06, 53 days at TH07, and 91

Table 5. 3: Means, standard deviation and range of the RILs of the cross ILC 588 x ILC 3279 and means for parental genotypes for harvest index (HI) and drought tolerance score (DTS) under field drought stress conditions during 2006 and 2007.

Trait ^a	Trial ^b	#	Parents		RILs					
			ILC 588	ILC 3279	Min ^c	Max	Mean	Se (+/-)	Skew	Kurt
HI	TH06	157	0.50	0.05	0.02	0.59	0.40	0.05	-1.4	1.9
	TH07	144	0.49	0.34	0.20	0.56	0.41	0.06	-0.5	-0.2
	BR07	110	0.24	0.10	0.06	0.33	0.19	0.03	0.0	-0.1
DTS	TH06	157	2.5	7.5	2.0	9.0	4.5	0.60	0.5	-0.3
	TH07	144	4.2	6.2	2.7	6.3	4.4	0.60	0.8	1.2
	BR07	110	4.1	6.3	2.8	6.8	4.3	0.49	0.6	0.6

^a HI, harvest index; DTS, Drought tolerance score on 1 to 9 scale where 1 is drought tolerant and 9 is drought susceptible

^b TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

^c Min, minimum; Max, maximum; se(+/-), standard error (+/-); Skew, skewness; Kurt, kurtosis

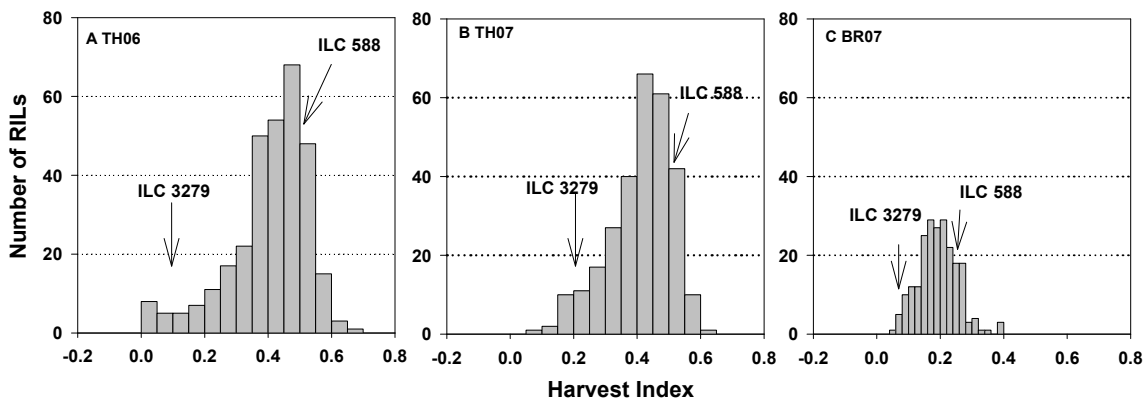


Fig. 5. 8: Frequency distribution of RILs of a cross of ILC 588 x ILC 3279 for harvest index for trials conducted at Tel Hadya, Syria during 2006 (A) and 2007 (B) and at Breda, Syria during 2007 (C). Arrows show the position of both parents of the population in the distribution.

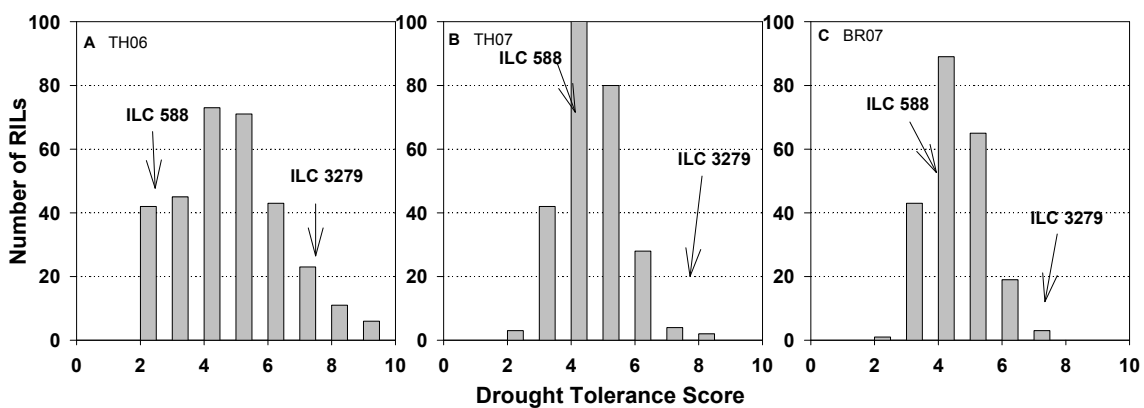


Fig. 5. 9: Frequency distribution of RILs of a cross of ILC 588 x ILC 3279 for drought tolerance score (DTS) for trials conducted at Tel Hadya, Syria during 2006 (A) and 2007 (B) and at Breda, Syria during 2007 (C), where 1 is drought tolerant and 9 is drought susceptible. Arrows show the position of both parents of the population in the distribution.

days at BR07. The number of days to maturity ranged from 72 to 111 at TH06, 87 to 99 days at TH07, and 139 to 154 days at BR07. The reason for the narrow range in maturity during TH07 could have been the abrupt rise in temperatures late in the growing season. Plant height was greater at BR07 as compared to the other two trials. This may have been due to the longer vegetative period and favourable growing conditions during the vegetative period at BR07. Minimum plant height (14 cm) occurred at TH06, while maximum plant height (47 cm) occurred at BR07.

5.4.7 Stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$)

Leaf stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) was measured at three dates, i.e., 7, 9 and 10 weeks after sowing (WAS) during TH06, and on a single date 11 WAS in TH07. The first date of measurement in 2006 corresponded with early flowering, while the last date of measurement during 2006 and 2007 corresponded with late flowering. Means, standard deviation and other statistical parameters are given in Table 5.5. The mean stomatal conductance at 7 WAS in TH06 was $369 \text{ mmol m}^{-2} \text{s}^{-1}$, increased at the second date of measurement to $517 \text{ mmol m}^{-2} \text{s}^{-1}$, and then decreased to $132 \text{ mmol m}^{-2} \text{s}^{-1}$ at the third date of measurement. Mean g_s at TH07 was $89 \text{ mmol m}^{-2} \text{s}^{-1}$ as measured 11 WAS.

The minimum and maximum values of g_s for the RILs also increased from the first date of measurement to the second date and then reduced at the third date of measurement. Mean g_s value of $132 \text{ mmol m}^{-2} \text{s}^{-1}$ at third date of measurement (10WAS)

Table 5.4: Means, standard deviation and range of the RILs of the cross ILC 588 x ILC 3279 and means for parental genotypes for various phenological traits under field drought stress conditions during 2006 and 2007.

Trait ^a	Trial ^b	#	Parents		RILs					
			ILC 588	ILC 3279	Min ^c	Max	Mean	Se (+/-)	Skew	Kurt
DFF	TH06	157	42	64	42	70	53	2	0.4	-0.1
	TH07	144	55	69	53	69	57	1	1.6	4.2
	BR07	110	98	102	91	104	95	2	0.7	-0.2
DM	TH06	157	77	111	72	111	90	3	0.5	-0.9
	TH07	144	88	99	87	99	90	1	1.0	2.2
	BR07	110	144	153	139	154	145	3	0.2	-0.7
RP	TH06	157	35	47	26	54	38	3	0.6	-0.4
	TH07	144	33	30	26	40	34	2	-0.8	1.9
	BR07	110	46	51	42	58	50	3	0.1	0.2
PH	TH06	157	23	30	14	38	26	2	-0.1	-0.3
	TH07	144	23	37	21	37	29	2	0.1	-0.9
	BR07	110	32	45	31	47	39	2	-0.1	-0.8

^a DFF, number of days from sowing to first flower (when 50% plants of line showed first flower); DM, number of days from sowing to physiological maturity; RP, number of days between flowering and physiological maturity; PH, plant height (cm)

^b TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

^c Min, minimum; Max, maximum; se(+/-), standard error (+/-); Skew, skewness; Kurt, kurtosis

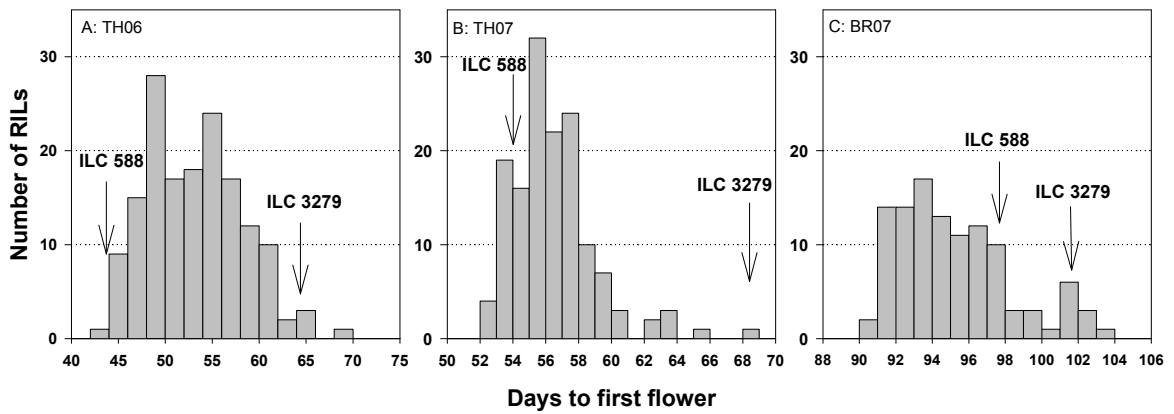


Fig. 5.10: Frequency distribution of RILs of a cross of ILC 588 x ILC 3279 for days to first flower for trials conducted at Tel Hadya, Syria during 2006 (A) and 2007 (B) and at Breda, Syria during 2007 (C). Arrows show the position of both parents of the population in the distribution.

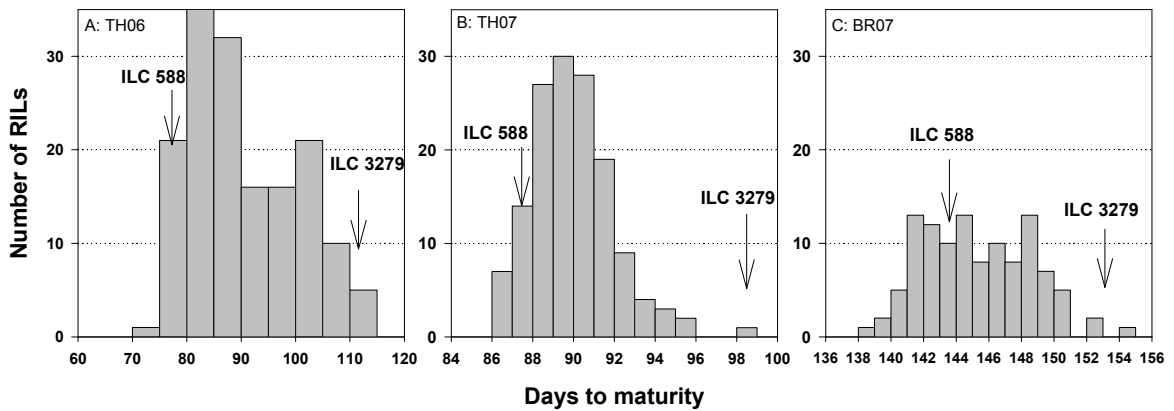


Fig. 5.11: Frequency distribution of RILs of a cross of ILC 588 x ILC 3279 for days to maturity for trials conducted at Tel Hadya, Syria during 2006 (A) and 2007 (B) and at Breda, Syria during 2007 (C). Arrows show the position of both parents of the population in the distribution.

during TH06 indicated that the plants were drought stressed. Similarly, mean g_s of 89 $\text{mmol m}^{-2} \text{s}^{-1}$ obtained at 11WAS during TH07 also indicated drought stress. The parental genotypes differed in terms of g_s at all dates of measurements. ILC 588 had g_s of 473 $\text{mmol m}^{-2} \text{s}^{-1}$ before flowering (7 WAS), 333 $\text{mmol m}^{-2} \text{s}^{-1}$ during flowering (9 WAS), and 124 $\text{mmol m}^{-2} \text{s}^{-1}$ at late-flowering (10 WAS). ILC 3279 had g_s of 354 $\text{mmol m}^{-2} \text{s}^{-1}$ at the first date of measurement, 269 $\text{mmol m}^{-2} \text{s}^{-1}$ at the second date of measurement, and further decreased to 66 $\text{mmol m}^{-2} \text{s}^{-1}$ at the third date of measurement. A similar pattern of g_s was observed in TH07 where ILC 588 showed g_s of 169 $\text{mmol m}^{-2} \text{s}^{-1}$, while ILC 3279 had g_s of 94 $\text{mmol m}^{-2} \text{s}^{-1}$. The values of g_s obtained on the third date of measurement (10WAS) during TH06 and 11WAS during TH07 indicated that the plants were drought stressed.

5.4.8 Canopy temperature

The difference between canopy temperature and air temperature ($T_c - T_a$) was measured at three dates during 7, 8 and 9 weeks after sowing (WAS) in TH06, at one date during 11 WAS in TH07, and at one date during 16 WAS in BR07. The first date of measurement during 2006 in TH06 corresponded to early flowering while last date of measurement during 2006 and 2007 in TH07 corresponded to late flowering. The date of measurement at BR07 corresponded to the flowering period. Means, standard deviation and other statistical parameters are given in Table 5.6. Canopy temperature

Table 5. 5: Means, standard deviation and range of the RILs of the cross ILC 588 x ILC 3279 and means for parental genotypes for stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) under field drought stress conditions during 2006 and 2007.

Trial ^a	Date	# of RILs	Parents		RILs					
			ILC 588	ILC 3279	Min ^b	Max	Mean	Se (+/-)	Skew	Kurt
TH06	7 WAS ^c	157	473	354	84	682	369	104	0.2	0.8
	9 WAS	157	333	269	148	896	517	154	-0.1	-0.5
	10 WAS	157	124	66	41	296	132	54	0.8	0.9
TH07	11 WAS	144	169	94	2	414	89	58	1.8	7.7

^a TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007

^b Min, minimum; Max, maximum; se(+/-), standard error (+/-); Skew, skewness; Kurt, kurtosis

^c WAS, weeks after sowing

Table 5. 6: Means, standard deviation and range of the RILs of the cross ILC 588 x ILC 3279 and means for parental genotypes for canopy temperature differential ($T_c - T_a$) under field drought stress conditions during 2006 and 2007.

Trial ^a	Date	# of RILs	Parents		RILs					
			ILC 588	ILC 3279	Min ^b	Max	Mean	Se (+/-)	Skew	Kurt
TH06	7 WAS ^c	157	0.6	3.4	-1.0	4.5	2.1	2.4	-0.3	-0.4
	8 WAS	157	-2.3	0.2	-3.3	1.7	-1.1	0.8	0.3	0.2
	9 WAS	157	-1.8	0.9	-2.4	1.1	-0.9	0.7	0.1	0.2
TH07	11 WAS	144	-1.7	-2.3	-3.2	1.0	-1.2	0.9	0.1	-0.4
BR07	16 WAS	110	0.2	1.1	-1.0	2.0	0.7	0.5	-0.1	-0.2

^a TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

^b Min, minimum; Max, maximum; se(+/-), standard error (+/-); Skew, skewness; Kurt, kurtosis

^c WAS, weeks after sowing

differential ($T_c - T_a$) ranged from $-0.9\text{ }^{\circ}\text{C}$ to $4.5\text{ }^{\circ}\text{C}$ at the first date of measurement at TH06, from $-3.3\text{ }^{\circ}\text{C}$ to $1.7\text{ }^{\circ}\text{C}$ at the second date, and from -2.4 to 1.1 at the third date. ILC 588 had canopy temperature differential at the lower end of the range at all trials with the exception of TH07. Similarly, ILC 3279 showed differential values at the higher end of the range at all the trials with the exception of TH07. As an example, the frequency distribution for canopy temperature differential ($T_c - T_a$) for the second date of measurement during 2007 is presented in Fig. 5.12. A normal distribution was detected, and the majority of RILs had lower canopy temperature than air temperature as did ILC 588, the drought tolerant parent.

5.4.9 Chlorophyll fluorescence parameters

Mean values and the related statistical parameters for chlorophyll fluorescence parameters are shown in Table 5.7. Parental genotypes were only significantly different on 10 WAS at TH07 for F_o and 10 WAS at TH06 for F_m . Similarly, variable fluorescence (F_v) differed for the parents only on 10 WAS at TH06 and 12 WAS at TH07. However, parents differed significantly for their maximum quantum efficiency of PSII (F_v/F_m) on all dates of measurement in three trials with the exception of 8 WAS at TH06. Phenotypic distribution of F_v/F_m on 6 WAS in TH06 was $0.34 - 0.77$ while on 10 WAS was $0.43 - 0.81$. The distribution in TH07 on 10 WAS was $0.65 - 0.82$ and on 12 WAS was $0.35 - 0.84$. The distribution at BR07 was $0.57 - 0.85$. Guo *et al.*, (2007) observed F_v/F_m in barley RILs in a range of $0.76 - 0.83$ under well-watered and $0.01 - 0.82$ under drought stressed greenhouse conditions. Bolhar-Nordenkamp *et al.* (1989) found F_v/F_m in the range of $0.75 - 0.85$ for non-stressed wheat plants.

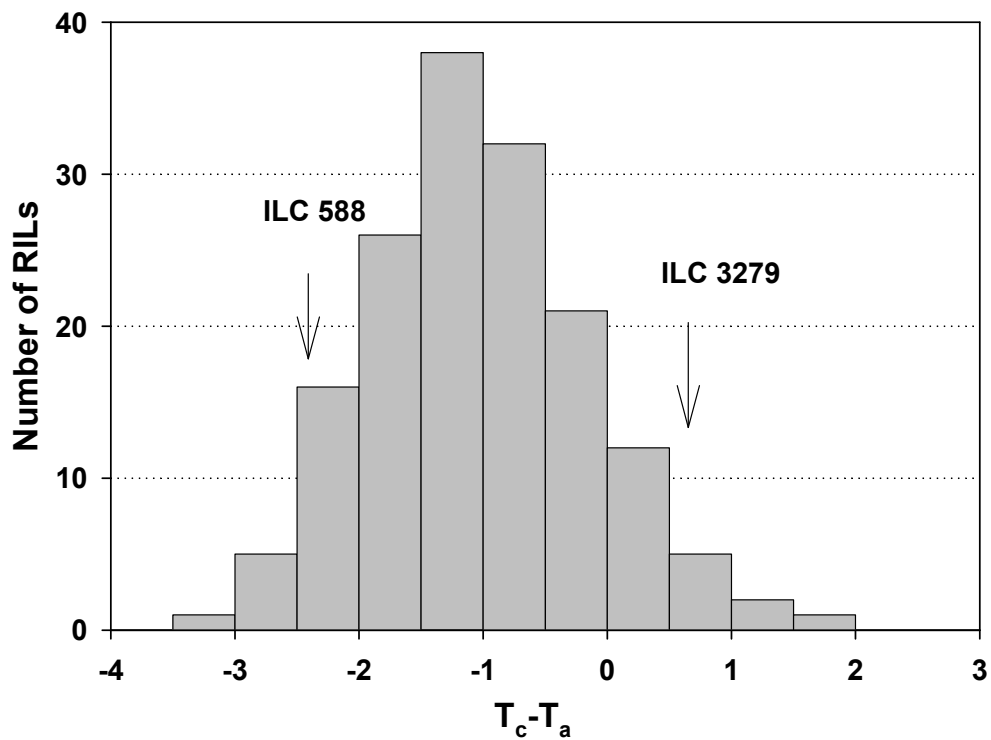


Fig. 5. 12: Frequency distribution of RILs of a cross of ILC 588 x ILC 3279 for canopy temperature differential ($T_c - T_a$) measured on 8 WAS at Tel Hadya, Syria during 2006. Arrows show the position of both parents of the population in the distribution.

Table 5. 7: Means, standard deviation and range of the RILs of the cross ILC 588 x ILC 3279 and means for parental genotypes for chlorophyll fluorescence parameters under field drought stress conditions during 2006 and 2007.

Trait ^a	Trial ^b	Date	# RILs	Parents		RILs					
				ILC 588	ILC 3279	Min ^c	Max	Mean	Se (+/-)	Skew	Kurt
Fo	TH06	6 WAS ^c	157	293	302	127	599	333	77	1.0	3.2
		8 WAS	157	230	226	188	517	345	75	0.2	-0.8
		10 WAS	157	310	321	128	566	304	63	0.7	1.8
	TH07	10 WAS	144	547	659	389	805	591	77	0.4	0.6
		12 WAS	144	554	545	332	2254	609	263	4.3	19.1
		BR07	110	769	854	414	1582	811	164	0.7	1.9
Fm	TH06	6 WAS	157	932	890	388	1093	913	79	-2.0	11.2
		8 WAS	157	1068	1047	729	1364	1082	122	-0.5	0.9
		10 WAS	157	897	1110	554	1228	1042	112	-1.1	2.5
	TH07	10 WAS	144	2235	2346	1664	2789	2308	257	-0.4	1.0
		12 WAS	144	2104	2458	1577	3131	2220	291	0.9	2.5
		BR07	110	3350	3437	2092	3768	3376	183	-2.2	7.9
Fv	TH06	6 WAS	157	639	589	261	714	580	73	-1.2	2.8
		8 WAS	157	838	822	410	905	737	135	-0.6	0.6
		10 WAS	157	587	789	329	958	737	107	-0.9	1.7
	TH07	10 WAS	144	1690	1688	1255	2108	1718	256	-0.2	0.1
		12 WAS	144	1542	1904	758	2039	1602	268	-1.2	1.6
		BR07	110	2574	2580	1649	2908	2561	190	-1.6	3.3
Fv/Fm	TH06	6 WAS	157	0.69	0.66	0.34	0.77	0.64	0.01	-1.5	4.3
		8 WAS	157	0.78	0.78	0.47	0.79	0.68	0.00	-0.4	0.1
		10 WAS	157	0.64	0.71	0.43	0.81	0.70	0.01	-0.2	2.8
	TH07	10 WAS	144	0.75	0.71	0.65	0.82	0.74	0.00	-0.6	0.3
		12 WAS	144	0.72	0.77	0.35	0.84	0.73	0.01	-2.9	9.9
		BR07	110	0.77	0.75	0.57	0.85	0.76	0.00	-1.0	2.8

^a Fo, initial fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidized; Fm, fluorescence level when Qa is transiently fully reduced, Fv, variable fluorescence (Fm-Fo); Fv/Fm, maximum quantum efficiency of photosystem II.

^b TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007;

BR07, trial location at Breda, Syria during 2007

^c Min, minimum; Max, maximum; se(+/-), standard error (+/-); Skew, skewness; Kurt, kurtosis

^c WAS, weeks after sowing

ILC 588 had higher Fv/Fm than ILC 3279 before flowering, equal at flowering and less after flowering. This may have been partially due to differences in maturity. The drought susceptible parent ILC 3279 matured 10-30 days later than ILC 588, and showed higher Fv/Fm than ILC 588 after flowering.

5.4.10 Heritability estimates

The heritability estimates and standard error of estimates for grain yield and yield components are provided in the Table 5.8, and those for important physiological and phenological traits are presented in the Table 5.9. The heritability was higher for grain yield (0.49 ± 0.03) and SDW (0.40 ± 0.04) at TH06 as compared to TH07 and BR07. Heritability estimates were higher for HI and DTS than for grain yield or SDW. Heritability estimates were higher at TH06 for HI (0.70 ± 0.01) and DTS (0.75 ± 0.01) as compared to the other trials. Similarly, heritability estimates were higher for DFF (0.71 ± 0.01) and DM (0.87 ± 0.002) at TH06 trial as compared to the other trials.

5.4.11 Phenotypic correlations among drought related traits

Phenotypic correlation coefficients among agronomic, morphological and physiological traits measured at the three trials are presented in Tables 5.10 – 5.16. Harvest index was positively correlated with grain yield at TH06 ($r=0.66$ $p<0.01$), TH07 ($r= 0.78$ $p<0.01$) and BR07 ($r= 0.82$ $p<0.01$). Drought tolerance score was negatively correlated with grain yield at TH06 ($r= -0.63$ $p<0.01$), TH07 ($r= -0.54$ $p<0.01$) and BR07 ($r= -0.26$ $p<0.01$). Days to flower showed a significant negative correlation with grain yield and harvest index and positive correlation with drought tolerance score in all three

Table 5. 8: Broad-sense heritability estimations for grain yield and yield components measured in three trials during 2006-07 for chickpea recombinant inbred lines of the cross ILC 588 x ILC 3279.

Trait ^a	Trial	σ_g^b	σ_e	$h^2 \pm se$
Grain Yield	TH06 ^c	0.77	0.79	0.49 ± 0.03
	TH07	0.75	1.00	0.43 ± 0.04
	BR07	0.23	0.48	0.32 ± 0.06
	Combined	0.23	0.79	0.41 ± 0.12
SDW	TH06	2.19	3.26	0.40 ± 0.04
	TH07	0.09	6.35	0.02 ± 0.08
	BR07	1.83	5.49	0.25 ± 0.07
	Combined	0.57	4.96	0.32 ± 0.14
100-GW	TH06	0.78	10.09	0.07 ± 0.07
	TH07	2.04	8.36	0.20 ± 0.07
	BR07	5.53	4.92	0.53 ± 0.03
	Combined	0.62	8.11	0.21 ± 0.14
Grain P ⁻¹	TH06	14.43	13.14	0.52 ± 0.03
	TH07	2.63	30.54	0.08 ± 0.08
	BR07	2.93	7.79	0.27 ± 0.07
	Combined	3.86	18.04	0.45 ± 0.13
Pods P ⁻¹	TH06	12.77	12.94	0.50 ± 0.03
	TH07	2.27	30.54	0.07 ± 0.08
	BR07	2.88	9.31	0.24 ± 0.07
	Combined	3.04	18.34	0.39 ± 0.13
Grain Pod ⁻¹	TH06	0.00	0.02	0.14 ± 0.07
	TH07	0.00	0.01	0.09 ± 0.08
	BR07	0.01	0.01	0.49 ± 0.04
	Combined	0.00	0.01	0.37 ± 0.12

^a Yield, Grain yield (g plant⁻¹); SDW, shoot dry weight (g plant⁻¹); 100-GW, hundred grain weight (g); Grain P⁻¹, number of grains per plant; Pods P⁻¹, number of pods per plant; Grain Pod⁻¹, number of grains per pod

^b σ_g , Genotypic variance; σ_e , environmental variance; $h^2 \pm se$, heritability estimates +/- standard error

^c TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

Table 5. 9: Broad-sense heritability estimations for important physiological and morphological traits measured in three trials during 2006-07 for chickpea recombinant inbred lines of the cross ILC 588 x ILC 3279.

Trait ^a	Environment	σ_g^b	σ_e	$h^2 \pm se$
HI	TH06 ^c	0.011	0.004	0.70 ± 0.01
	TH07	0.003	0.007	0.31 ± 0.05
	BR07	0.002	0.002	0.56 ± 0.03
	Combined	0.004	0.004	0.65 ± 0.09
DTS	TH06	2.15	0.72	0.75 ± 0.01
	TH07	0.26	0.55	0.32 ± 0.05
	BR07	0.44	0.43	0.51 ± 0.04
	Combined	0.43	0.59	0.52 ± 0.10
DFF	TH06	20.10	8.06	0.71 ± 0.01
	TH07	4.71	3.30	0.59 ± 0.02
	BR07	7.01	4.40	0.61 ± 0.02
	Combined	7.47	5.41	0.71 ± 0.07
DM	TH06	89.03	13.08	0.87 ± 0.002
	TH07	2.02	3.78	0.35 ± 0.05
	BR07	5.58	10.65	0.34 ± 0.06
	Combined	13.27	9.21	0.49 ± 0.11
PH	TH06	15.60	6.90	0.69 ± 0.01
	TH07	10.02	4.95	0.67 ± 0.02
	BR07	12.03	5.18	0.70 ± 0.02
	Combined	11.06	5.74	0.85 ± 0.04

^a HI, harvest index; DTS, drought tolerance score on 1-9 scale; DFF, number of days from sowing to first flower (when 50% plants of line showed first flower); DM, number of days from sowing to physiological maturity; PH, plant height (cm)

^b σ_g , Genotypic variance; σ_e , environmental variance; $h^2 \pm se$, heritability estimates +/- standard error

^c TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

trials with the exception of non-significant correlation with grain yield at BR07 trial which had a longer growth period (due to early sowing in January), early cold period and high degree of drought and high temperature at the flowering and post flowering periods. Days to maturity was also not correlated with grain yield at TH07 which again might have been due to high temperature which caused plants to mature early. Shoot dry weight (SDW), 100-grain weight (g), number of grains plant⁻¹, number of pods plant⁻¹ and number of grains pod⁻¹ were also generally positively correlated with grain yield and harvest index and negatively correlated with drought tolerance score.

Stomatal conductance showed a weak but positive correlation with grain yield and harvest index at 9 WAS at TH06 and at 11WAS at TH07. Canopy temperature differential showed a stronger correlation with grain yield and harvest index in all the trials with the exception of BR07. The relationship of chlorophyll fluorescence parameters with agronomic traits was somewhat inconsistent in the three trials.

5.4.12 Relationship of traits with yield performance under drought

The means and ranges for important agronomic and morphological traits for the population, for a subset of drought tolerant RILs ('Group A' which includes 17 RILs with above average grain yield in all three trials) and for a subset of drought susceptible RILs ('Group B' which includes 42 RILs with below average grain yield in all three trials) is shown in Table 5.17, while data for stomatal conductance is presented in Table 5.18, for Tc-Ta in the Table 5.19, and for maximum photosynthetic efficiency of PSII in Table 5.20. The same genotypes were included in group A and B for all the data presented.

Table 5. 10: Phenotypic correlation coefficients among agro-morphological traits measured on a population of RILs from a cross between ILC 588 x ILC 3279 under drought (TH06) at Tel Hadya, Syria during 2006.

Traits ^a	HI	DTS	DFE	DM	RP	PH	SDW	100-GW	Grain P ⁻¹	Pods P ⁻¹	Grain Pod ⁻¹
GY	0.66**	-0.63**	-0.47**	-0.51**	-0.42**	-0.14	0.70**	0.15	0.97**	0.94**	0.15*
HI		-0.84**	-0.78**	-0.82**	-0.65**	-0.60**	-0.03	0.08	0.65**	0.70**	-0.05
DTS			0.84**	0.93**	0.78**	0.51**	-0.06	0.02	-0.65**	-0.72**	0.12
DFE				0.84**	0.50**	0.50**	0.10	-0.03	-0.47**	-0.55**	0.13
DM					0.90**	0.55**	0.07	0.08	-0.54**	-0.62**	0.19*
RP						0.46**	0.04	0.15*	-0.46**	-0.53**	0.19*
PH							0.39**	-0.02	-0.14	-0.20**	0.17*
SDW								0.18*	0.66**	0.59**	0.29**
100-GW									-0.08	-0.07	0.10
Grain P ⁻¹										0.97**	0.17*
Pods P ⁻¹											-0.01

^a GY, grain yield (g plant⁻¹); HI, harvest index; DTS, drought tolerance score on 1-9 scale; DFE, days from sowing to first flower; DM, days from sowing to maturity; RP, days between time of first flower and physiological maturity; PH, plant height (cm); SDW, shoot dry weight (g plant⁻¹); 100-GW, hundred grain weight (g); Grain P⁻¹, number of grains per plant; Pods P⁻¹, number of pods per plant; Grain Pod⁻¹, number of grains per pod

Table 5. 11: Phenotypic correlation coefficients among agro-physiological traits measured on a population of RILs from a cross between ILC 588 x ILC 3279 under drought (TH06) at Tel Hadya, Syria during 2006.

Traits ^a	SC			Tc-Ta		
	7WAS ^b	9WAS	10WAS	7WAS	8WAS	9WAS
GY	0.06	0.18*	0.08	-0.25**	-0.36**	-0.35**
HI	-0.03	0.19*	0.14	-0.27**	-0.22**	-0.17*
DTS	0.06	-0.13	-0.08	0.34**	0.34**	0.11
DFE	0.04	-0.02	0.06	0.36**	0.38**	0.09
DM	0.05	-0.09	-0.06	0.32**	0.26**	-0.02
SC	7WAS	0.14	0.14	-0.04	-0.01	-0.17*
	8WAS		0.25**	-0.06	-0.07	-0.26**
	10WAS		-0.02	0.15	-0.17*	
Tc-Ta	7WAS				0.53**	0.12
	8WAS					0.28**

^a GY, grain yield (g plant⁻¹); HI, harvest index; DTS, drought tolerance score on 1-9 scale; DFE, days from sowing to first flower; DM, days from sowing to maturity; SC, stomatal conductance (g_s, mmol m⁻² s⁻¹); Tc-Ta, canopy temperature differential.

^b WAS, weeks after sowing

Table 5. 12: Phenotypic correlation coefficients among agro-physiological traits measured on a population of RILs from a cross between ILC 588 x ILC 3279 under drought (TH06) at Tel Hadya, Syria during 2006.

Traits	Fo			Fm			Fv			Fv/Fm		
	6WAS	8WAS	10WAS	6WAS	8WAS	10WAS	6WAS	8WAS	10WAS	6WAS	8WAS	10WAS
GY	0.10	0.02	0.00	0.11	0.09	-0.09	0.04	0.09	-0.10	-0.04	0.03	-0.05
HI	0.04	0.07	-0.07	0.04	0.05	-0.32**	0.01	0.00	-0.28**	0.00	-0.07	-0.08
DTS	-0.06	-0.03	-0.04	-0.11	-0.11	0.28**	-0.06	-0.11	0.31**	-0.02	-0.03	0.17*
DFE	0.00	-0.03	-0.03	-0.08	-0.15	0.31**	-0.09	-0.15	0.33**	-0.06	-0.04	0.19*
DM	-0.01	-0.03	-0.01	-0.05	-0.10	0.34**	-0.04	-0.09	0.35**	-0.04	-0.01	0.18*
Fo	6WAS ^b	-0.19*	0.26**	0.47**	-0.06	0.03	-0.36**	0.08	-0.14	-0.79**	0.17*	-0.28**
	8WAS		-0.27**	-0.09	0.55**	-0.26**	0.06	-0.16*	-0.09	0.16*	-0.85**	0.11
	10WAS			0.12	-0.21**	0.34**	-0.10	-0.03	-0.29**	-0.24**	0.19*	-0.81**
Fm	6WAS				0.16*	0.03	0.65**	0.26**	-0.05	0.13	0.19*	-0.11
	8WAS					-0.17*	0.22**	0.74**	-0.04	0.16*	-0.06	0.11
	10WAS						0.01	0.01	0.81**	-0.04	0.21**	0.24**
Fv	6WAS							0.21**	0.07	0.82**	0.06	0.13
	8WAS								0.03	0.06	0.62**	0.04
	10WAS									0.11	0.09	0.76**
Fv	6WAS										-0.08	0.25**
/Fm	8WAS											-0.07

^a GY, grain yield (g plant⁻¹); HI, harvest index; DTS, drought tolerance score on 1-9 scale; DFE, days from sowing to first flower; DM, days from sowing to maturity; Fo, initial fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidized; Fm, fluorescence level when Qa is transiently fully reduced, Fv, variable fluorescence (Fm-Fo); Fv/Fm, maximum quantum efficiency of photosystem II.

^b WAS, weeks after sowing

Table 5. 13: Phenotypic correlation coefficients among agro-morphological traits measured on a population of RILs from a cross between ILC 588 x ILC 3279 under drought (TH07) at Tel Hadya, Syria during 2007.

Traits ^a	HI	DTS	DFE	DM	RP	PH	SDW	100-GW	Grain P ⁻¹	Pods P ⁻¹	Grain Pod ⁻¹
GY	0.78**	-0.54**	-0.26**	-0.05	0.26**	0.01	0.76**	0.63**	0.90**	0.86**	0.26**
HI		-0.56**	-0.38**	-0.19*	0.27**	-0.17*	0.29**	0.68**	0.66**	0.62**	0.24**
DTS			0.55**	0.21**	-0.45**	-0.06	-0.39**	-0.39**	-0.55**	-0.52**	-0.15
DFE				0.57**	-0.64**	0.24**	-0.06	-0.18*	-0.25**	-0.23**	-0.12
DM					0.26**	0.28**	0.11	0.06	-0.08	-0.07	-0.07
RP						-0.02	0.18*	0.28**	0.22**	0.21**	0.08
PH							0.25**	0.07	0.04	0.07	-0.13
SDW								0.34**	0.84**	0.82**	0.20*
100-GW									0.34**	0.33**	0.15
Grain P ⁻¹										0.96**	0.27**
Pods P ⁻¹											0.01

^a GY, grain yield (g plant⁻¹); HI, harvest index; DTS, drought tolerance score on 1-9 scale; DFE, days from sowing to first flower; DM, days from sowing to maturity; RP, days between time of first flower and physiological maturity; PH, plant height (cm); SDW, shoot dry weight (g plant⁻¹); 100-GW, hundred grain weight (g); Grain P⁻¹, number of grains per plant; Pods P⁻¹, number of pods per plant; Grain Pod⁻¹, number of grains per pod

Table 5. 14: Phenotypic correlation coefficients among agro-physiological traits measured on a population of RILs from a cross between ILC 588 x ILC 3279 under drought (TH07) at Tel Hadya, Syria during 2007.

Traits ^a	SC	Tc-Ta	Fo		Fm		Fv		Fv/Fm	
	11WAS	11WAS	10WAS	12WAS	10WAS	12WAS	10WAS	12WAS	10WAS	12WAS
GY	0.27**	-0.41**	-0.16*	-0.22**	-0.04	0.00	0.03	0.28**	0.11	0.27**
HI	0.33**	-0.34**	-0.25**	-0.17*	-0.07	-0.06	0.04	0.20*	0.18*	0.20**
DTS	0.01	0.14	0.26**	0.13	-0.05	-0.02	-0.17*	-0.12	-0.30**	-0.17*
DFF	-0.03	-0.10	0.30**	-0.01	0.07	0.10	-0.06	0.14	-0.23**	0.05
DM	0.01	-0.21**	0.20**	-0.20*	-0.06	-0.01	-0.15	0.33**	-0.18*	0.25**
SC	11WAS ^b	-0.21**	-0.16*	-0.09	-0.10	-0.14	-0.04	0.01	0.10	0.09
Tc-Ta	11WAS		0.04	0.17*	0.12	0.08	0.10	-0.18*	0.03	-0.18*
Fo	10WAS			0.11	0.32**	0.09	-0.12	0.13	-0.78**	-0.05
	12WAS				-0.01	0.36**	-0.06	-0.57**	-0.11	-0.90**
Fm	10WAS					0.03	0.90**	0.05	0.28**	0.05
	12WAS						-0.02	0.13	-0.08	-0.20**
Fv	10WAS							-0.02	0.66**	0.07
	12WAS								-0.09	0.79**
Fv/Fm	10WAS									0.08

^a GY, grain yield (g plant⁻¹); HI, harvest index; DTS, drought tolerance score on 1-9 scale; DFF, days from sowing to first flower; DM, days from sowing to maturity; SC, stomatal conductance (g_s, mmol m⁻² s⁻¹); Tc-Ta, canopy temperature differential; Fo, initial fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidized; Fm, fluorescence level when Qa is transiently fully reduced, Fv, variable fluorescence (Fm-Fo); Fv/Fm, maximum quantum efficiency of photosystem II

^b WAS, weeks after sowing

Table 5. 15: Phenotypic correlation coefficients among agro-morphological traits measured on a population of RILs from a cross between ILC 588 x ILC 3279 under drought (BR07) at Breda, Syria during 2007.

Traits ^a	HI	DTS	DFE	DM	RP	PH	SDW	100-GW	Grain P ⁻¹	Pods P ⁻¹	Grain Pod ⁻¹
GY	0.82**	-0.26**	-0.07	-0.03	0.04	-0.27**	0.50**	0.62**	0.93**	0.81**	0.46**
HI		-0.39**	-0.43**	-0.22*	0.19*	-0.46**	-0.05	0.61**	0.73**	0.51**	0.56**
DTS			0.48**	0.35**	-0.10	0.14	0.15	-0.09	-0.27**	-0.18	-0.23**
DFE				0.55**	-0.39**	0.32**	0.54**	0.01	-0.10	0.11	-0.35**
DM					0.55**	0.15	0.31**	0.03	-0.03	0.10	-0.23**
RP						-0.16	-0.20*	0.02	0.06	-0.01	0.10
PH							0.22*	-0.29**	-0.20*	-0.08	-0.21*
SDW								0.18	0.52**	0.70**	-0.05
100-GW									0.31**	0.33**	0.05
Grain P ⁻¹										0.83**	0.56**
Pods P ⁻¹											0.03

^a GY, grain yield (g plant⁻¹); HI, harvest index; DTS, drought tolerance score on 1-9 scale; DFE, days from sowing to first flower; DM, days from sowing to maturity; RP, days between time of first flower and physiological maturity; PH, plant height (cm); SDW, shoot dry weight (g plant⁻¹); 100-GW, hundred grain weight (g); Grain P⁻¹, number of grains per plant; Pods P⁻¹, number of pods per plant; Grain Pod⁻¹, number of grains per pod

Table 5. 16: Phenotypic correlation coefficients among agro-physiological traits measured on a population of RILs from a cross between ILC 588 x ILC 3279 under drought (BR07) at Breda, Syria during 2007.

Traits ^a	Tc-Ta	Fo	Fm	Fv	Fv/Fm
	16WAS ^b	16WAS	16WAS	16WAS	16WAS
GY	0.01	0.00	0.12	0.14	0.06
HI	0.12	-0.03	0.04	0.07	0.06
DTS	0.17	0.06	0.05	0.01	-0.06
DFF	-0.18	0.13	0.17	0.09	-0.08
DM	-0.06	0.12	0.17	0.10	-0.09
Tc-Ta		0.03	-0.05	-0.09	-0.05
Fo			0.56**	-0.22**	-0.93**
Fm				0.68**	-0.23*
Fv					0.54**

^a GY, grain yield (g plant⁻¹); HI, harvest index; DTS, drought tolerance score on 1-9 scale; DFF, days from sowing to first flower; DM, days from sowing to maturity; Tc-Ta, canopy temperature differential; Fo, initial fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidized; Fm, fluorescence level when Qa is transiently fully reduced, Fv, variable fluorescence (Fm-Fo); Fv/Fm, maximum quantum efficiency of photosystem II

^b WAS, weeks after sowing

Differences between Group A and Group B for grain yield (g plant^{-1}), harvest index, drought tolerance score (DTS), shoot dry weight (g plant^{-1}), days to flowering, days to maturity, 100-grain weight and final plant height (cm) were evident. The data suggested that high harvest index, early flowering, early maturity and high 100-grain weight were associated with higher grain yield under drought. Similarly, Table 5.18 revealed that RILs in Group A had higher stomatal conductance than Group B over three dates of measurement at TH06 and TH07. Stomatal conductance values ($g_s < 172 \text{ mmol m}^{-2} \text{ s}^{-1}$) at 10WAS during TH07 and 11WAS during TH07 indicated drought stress. Comparatively higher g_s of Group A compared to Group B indicated a better transpiration rate. Similarly, Table 5.19 revealed that RILs in Group A had cooler canopies than Group B over three dates of measurement at TH06, TH07 and BR07. Genotypes showing positive canopy temperature differential could not maintain adequate transpiration rate and hence, transpirational cooling was reduced. These results suggest that higher stomatal conductance and cooler canopies were associated with better performance under drought conditions in Mediterranean environments. Maximum photosynthetic efficiency of PSII system (F_v/F_m) did not differ between the means of the two groups (Table 5.20). Differences for chlorophyll fluorescence parameters on dark adapted leaves were not detected between drought tolerant and susceptible RILs in this population.

Table 5. 17: Means with standard error and ranges of the data for important agronomic and morphological traits of chickpea population ILC 588 x ILC 3279 at three environments under natural drought in Syria during 2006 and 2007.

Category	RILs	Yield ^a	HI	DTS	SDW	DFE	DM	100-GW	PH
TH06^b									
Population	157	2.8 ± 0.6 (0.1-6.0)*	0.40 ± 0.05 (0.02-0.59)	4.5 ± 0.6	7.0 ± 1.3	53 ± 2.0 (42-70)	90 ± 2.7 (72-111)	23.5 ± 2.0 (15-32)	26 ± 2
Group A ^φ	17	3.8 (2.8-5.2)	0.48 (0.34-0.58)	3.3 (2.0-5.5)	8.0 (6.1-11.1)	50 (42-57)	84 (76-104)	24.3 (20-32)	24 (18-33)
Group B ^c	42	2.0 (0.1-4.7)	0.34 (0.02-0.50)	5.40 (2.5-9.0)	6.0 (3.4-11.1)	55 (46-70)	95 (79-111)	22.6 (15-26)	28 (19-36)
TH07									
Population	157	3.8 ± 0.7 (0.8-6.9)	0.41 ± 0.06 (0.20-0.56)	4.4 ± 0.6	9.2 ± 1.8	56 ± 1.3 (53-69)	90 ± 1.4 (87-99)	22.7 ± 2.1 (17-29)	29 ± 2
Group A	17	4.8 (3.8-6.9)	0.47 (0.35-0.55)	4.1 (3.2-4.8)	10.4 (8.3-14.4)	55 (53-59)	89 (87-93)	24.1 (19-28)	27 (23-33)
Group B	42	3.8 (0.8-5.2)	0.37 (0.20-0.51)	4.83 (3.8-7.2)	8.1 (4.7-14.7)	58 (54-69)	91 (87-99)	21.4 (17-27)	29 (23-37)
BR07									
Population	157	2.1 ± 0.5 (0.6-4.2)	0.19 ± 0.03 (0.06-0.33)	4.3 ± 0.5	10.5 ± 1.7	95 ± 1.5 (91-104)	145 ± 2.5 (139-154)	20.7 ± 1.6 (13-26)	39 ± 2
Group A	17	2.6 (2.2-4.2)	0.24 (0.16-0.33)	3.9 (2.8-5.4)	10.9 (8.1-17.3)	94 (91-100)	143 (140-149)	22.3 (19-26)	38 (32-46)
Group B	42	1.5 (0.6-2.9)	0.15 (0.06-0.24)	4.6 (2.9-6.8)	9.4 (5.6-13.8)	96 (92-104)	146 (141-153)	19.3 (14-26)	41 (34-47)

^a Yield, Grain yield (g plant⁻¹); HI, harvest index; DTS, Drought tolerance score on 1 to 9 scale where 1 is drought tolerant and 9 is drought susceptible; SDW, shoot dry weight (g plant⁻¹); DFE, number of days from sowing to first flower (when 50% plants of line showed first flower); DM, number of days from sowing to physiological maturity; 100-GW, hundred grain weight (g); PH, plant height (cm)

^b TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

* Values in the brackets show the range of the data. ^φ Group A includes 17 RILs which had above average mean grain yield in each trial. ^c Group B includes 42 RILs which had below average mean grain yield in each trial.

Table 5. 18: Means with standard error and range of the data for stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) of chickpea population ILC 588 x ILC 3279 under natural drought at Tel Hadya, Syria in 2006 and 2007.

Category	RILs	2006			2007
		7WAS ^c	8WAS	10WAS	11WAS
Population	157	369 ± 104 (138 – 722)*	518 ± 154 (162 – 903)	132 ± 54 (29 – 298)	89 ± 58 (2 – 414)
Group A ^φ	17	387 (262 – 722)	584 (418 – 885)	172 (96 – 275)	130 (61 – 414)
Group B ^c	42	333 (137 – 517)	422 (162 – 645)	116 (48 – 166)	71 (2 – 129)

* Values in the brackets show the range of the data for that category. ^φ Group A includes 17 RILs which had above average mean grain yield. ^c Group B includes 42 RILs which had below average mean grain yield.

^c WAS, weeks after sowing

Table 5. 19: Means with standard error and range of the data for canopy temperature differential (Tc-Ta) of chickpea population ILC 588 x ILC 3279 under natural drought during 2006 & 2007 at two locations in Syria.

Category	RILs	Tel Hadya				Breda
		2006			2007	2007
		7WAS ^c	8WAS	9WAS	11WAS	16WAS
Population	157	2.2 ± 2.4 (-1.0 – 4.5)	-1.0 ± 0.8 (-3.3 – 1.7)	-0.9 ± 0.8 (-2.4 – 1.1)	-1.2 ± 0.9 (-3.2 – 1.0)	1.1 ± 0.5 (-0.6 – 2.4)
Group A ^φ	17	1.1 (-1.0 – 2.3)	-1.7 (-2.9 – -0.2)	-1.1 (-1.8 – 0.1)	-1.6 (-3.1 – 0.4)	1.2 (-0.1 – 2.0)
Group B ^c	42	2.4 (-0.7 – 4.2)	0.7 (-2.1 – 1.7)	-0.7 (-1.9 – 1.1)	-1.0 (-2.8 – 0.8)	1.1 (0.2 – 2.1)

* Values in the brackets show the range of the data. ^φ Group A includes 17 RILs which had above average mean grain yield. ^c Group B includes 42 RILs which had below average mean grain yield.

^c WAS, weeks after sowing

Table 5. 20: Means with standard error and range of the data for Fv/Fm of chickpea population ILC 588 x ILC 3279 under natural drought during 2006 and 2007 at two locations in Syria.

Category	RILs	Tel Hadya					Breda
		2006		2007			2007
		6WAS ^c	8WAS	10WAS	10WAS	12WAS	16WAS
Population	157	0.64 ± 0.07 (0.34 – 0.77)	0.68 ± 0.08 (0.47 – 0.79)	0.70 ± 0.06 (0.43 – 0.81)	0.74 ± 0.04 (0.65 – 0.82)	0.73 ± 0.07 (0.35 – 0.84)	1.31 ± 0.6 (1.20 – 1.40)
Group A ^o	17	0.65 (0.50 – 0.77)	0.70 (0.62 – 0.79)	0.70 (0.55 – 0.81)	0.75 (0.71 – 0.80)	0.74 (0.61 – 0.84)	1.33 (1.27 – 1.39)
Group B ^c	42	0.62 (0.34 – 0.69)	0.68 (0.51 – 0.78)	0.69 (0.43-0.77)	0.73 (0.67 – 0.78)	0.71 (0.35 – 0.81)	1.31 (1.26 – 1.39)

* Values in the brackets show the range of the data. ^o Group A includes 17 RILs which had above average mean grain yield.

^c Group B includes 42 RILs which had below average mean grain yield.

^c WAS, weeks after sowing

5.4.12 Genetic Linkage Map

5.4.12.1 Polymorphism and markers for mapping

A total of 260 SSR primer pairs obtained from Hüttel *et al.* (1999), Winter *et al.* (1999), Sethy *et al.* (2003) and Lichtenzweig *et al.* (2005) were tested for polymorphism between the parents ILC 588 and ILC 3279. A total of 110 (42%) primer pairs revealed DNA polymorphism between the parents. The RIL population was screened with 54 of these primer pairs and a genetic linkage map (Fig. 5.13) was developed using Mapmaker/Exp and Windows QTL Cartographer. The map consisted of eight linkage groups plus two unlinked loci.

5.4.12.2 Segregation distortion analysis

The goodness-of-fit of the observed segregation ratio to the expected ratio (chi-square test, $P < 0.05$) identified 11 (19%) loci that did not segregate in accordance with the expected Mendelian inheritance ratio of 1:1 (Table 5.21). The majority of the loci with distorted segregation ratios were located on LG5 (H4F02, TR59, TR18, H3H07, TA39, TA5 and TS35) while LG2 (H4A04), LG3 (TA125) and LG6 (TA14) each had one locus with distorted segregation ratios.

5.4.12.3 General features of the map

The general features of the intraspecific map of chickpea are summarized in Table 5.22. A total of 52 SSR markers were mapped into eight linkage groups that spanned 334.8 cM of the chickpea genome at an average density of 6.4 cM. LG1 represented the smallest linkage group in terms of size and number of markers mapped. There were four

markers spanning only 2.9 cM with an average marker density of 0.7 cM. On the other hand, LG6 spanned 91.8 cM with eight loci and an average marker density of 11.5 cM. Linkage groups LG1, LG2 and LG5 were the most dense with an average marker density less than 3.0 cM. Linkage groups LG3, LG4, LG6, LG7 and LG8 showed dense sub-clusters either at the central region or at distal ends.

Markers in common between this map and the map produced by Winter *et al.* (2000) are underlined while common markers with the map produced by Tar'an *et al.* (2007) are bolded in Fig. 5.13. A total of 44 markers out of 52 were common with one or both of these two maps allowing alignment and naming of linkage groups of this map.

5.4.13 Mapping QTL associated with drought tolerance

QTLs for agronomic, morphological and physiological traits measured under natural drought stress in three field trials during 2006 and 2007 were identified and are presented in Tables 5.23 - 5.25.

A total of 13 genomic regions were identified as having significant association with various drought related traits under field conditions (Fig. 5.13). The contribution of individual QTL in terms of phenotypic variability of traits (% explanation, R^2) and additive effect for each QTL are also presented in Tables 5.23 - 5.25. Positive values of additive effect indicate the donor of the allele for the trait was ILC 588 while negative values indicate the donor of the allele for the trait was ILC 3279. Almost the whole of LG1, between markers H5A08 and TA8 (2.5 cM), was associated with various drought related traits viz: grain yield under drought, harvest index (HI), drought tolerance score (DTS), days to first flower, days to physiological maturity, reproductive period, plant

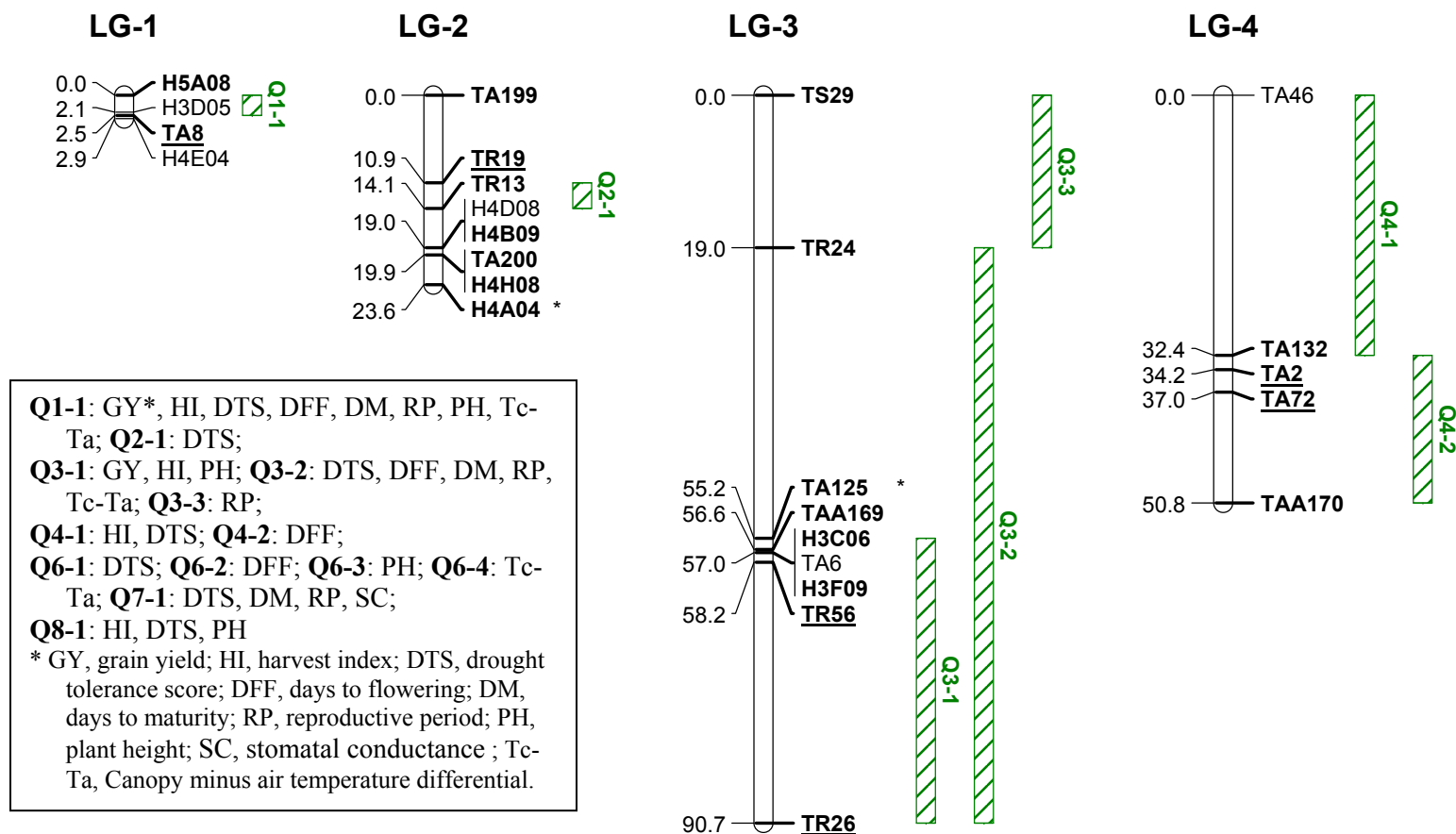


Fig. 5. 13: Genetic linkage map of chickpea (*Cicer arietinum* L.) developed from 52 SSR markers based on 128 RILs developed from an intraspecific cross between ILC 588 and ILC 3279. Linkage distance (cM) is indicated on the left of each linkage group. Markers with asterisk (*) are deviated from 1:1 Mendelian segregation ratio at $P=0.05$. Markers in bold are common on the same linkage group with genetic map of ICCV 96029 (desi) x CDC Frontier (kabuli) (Tar'an *et al.*, 2007), while markers in underline are common with genetic map of *C. arietinum* (ICC-4958) x *C. reticulatum* (PI 4897777) (Winter *et al.*, 2000). Vertical bars indicate the location of QTLs corresponds to traits as mentioned in the box. Only QTLs significant than threshold are shown.

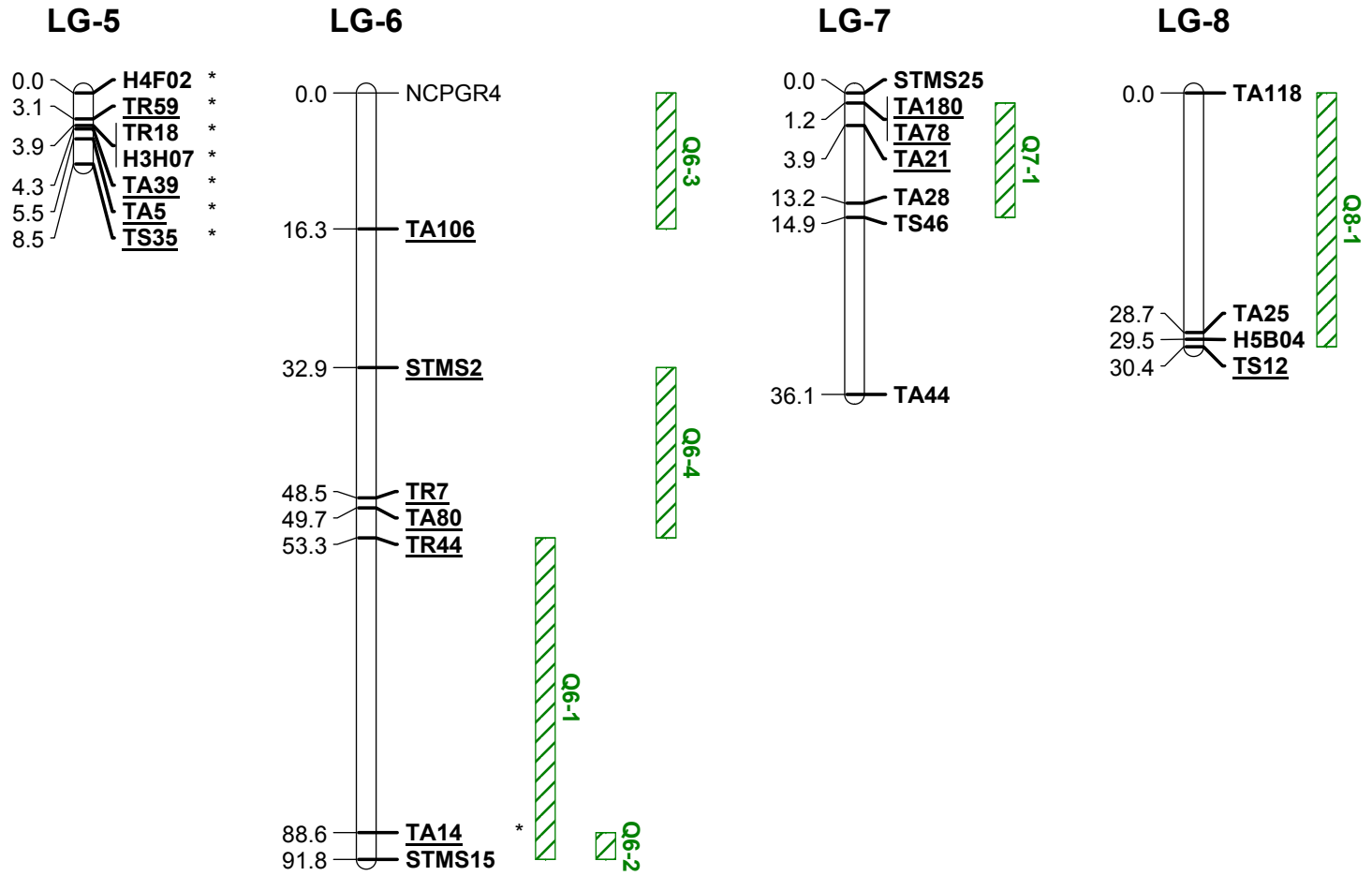


Fig. 5.13: continued

Table 5. 21: Segregation ratios of chickpea SSR markers that deviated from the expected 1:1 Mendelian ratio and frequency of maternal alleles in the mapping population. Segregation distortion ratio (χ^2 at P<0.05) is 19%.

Marker	Linkage group	Segregation ratios		Chi-square (P<0.05) ^a	Frequency of maternal alleles (%)
		Expected	Observed		
H4A04	LG2	52:52	42:62	3.9	60
TA125	LG3	58:58	74:41	9.5	36
H4F02	LG5	61:61	79:43	10.6	35
H3H07	LG5	64:64	80:48	8.0	38
TA39	LG5	63:63	79:47	8.1	37
TA5	LG5	62:62	74:50	4.7	40
TR18	LG5	64:64	80:47	8.6	37
TR59	LG5	64:64	82:45	10.8	35
TS35	LG5	64:64	82:45	10.8	35
TA14	LG6	64:64	80:47	8.6	37
Mean (Range)				8.3(3.9-10.8)	39(35-60)

^a $\chi^2_{(0.05,1)}=3.84$

Table 5. 22: General features of genetic map of chickpea (*Cicer arietinum* L.) developed using SSR markers based on 128 RILs population developed from an intraspecific cross between ILC 588 and ILC 3279.

Linkage group (LG)	Size (cM)	Number of mapped markers			Average marker density (cM)
		Mendelian segregation	Distorted segregation	Total	
LG1	2.9	4	0	4	0.7
LG2	23.6	7	1	8	3.0
LG3	90.7	8	1	9	10.1
LG4	50.8	5	0	5	10.2
LG5	8.5	0	7	7	1.2
LG6	91.8	7	1	8	11.5
LG7	36.1	7	0	7	5.2
LG8	30.4	4	0	4	7.6
Total/Ave.	334.8	42	10	52	6.4

Table 5. 23: Quantitative trait loci detected for grain yield plant⁻¹ (GY), harvest index (HI) and drought tolerance score (DTS) at one location in 2006 and two locations in 2007.

Trait ^a	Trial ^b	Linkage group	Interval	Interval length (cM)	Nearest locus to the maximum LOD peak	Threshold LOD Score*	Maximum LOD score	Additive effects [^]	% Explanation (R ²)
GY	TH06	LG1	H5A08-TA8	2.5	H3D05	2.3	2.9	-0.33	8
	TH07	LG1	H5A08-TA8	2.5	H3D05	2.4	2.8	-0.38	11
	TH06	LG3	TA125-TR26	37.0	TR56	2.3	3.0	-0.45	16
HI	TH06	LG1	H5A08-TA8	2.5	TA8	2.1	6.2	-0.04	13
	TH07	LG1	H5A08-TA8	2.5	H3D05	2.1	2.6	-0.03	11
	TH06	LG3	TA125-TR26	37.0	TR56	2.1	9.9	-0.06	29
	TH06	LG4	TA46-TA132	32.0	TA132	2.1	2.5	-0.04	10
	BR07	LG8	TA118-TS12	30.0	TA25	2.3	2.7	-0.03	23
DTS	TH06	LG1	H5A08-TA8	2.5	TA8	2.3	5.3	0.53	10
	BR07	LG2	TR19-TR13	3.0	TR19	2.1	2.3	-0.26	10
	TH06	LG3	TR24-TR26	69.0	TR56	2.3	12.9	1.02	35
	TH06	LG4	TA46-TA132	32.0	TA132	2.3	2.4	0.50	9
	BR07	LG6	TR44-STMS15	37.0	TA14	2.4	2.8	0.32	14
	TH06	LG7	TA180-TS46	15.0	TA21	2.3	2.7	0.38	5
	BR07	LG8	TA118-TS12	30.0	TA25	2.1	5.0	0.48	33

^a GY, grain yield plant⁻¹; HI, harvest index; DTS, drought tolerance score

^b TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

* Threshold level to declare a QTL significant was determined by performing 1000 permutation of the data by maintaining the type I error rate of 0.05

[^] Negative values indicated allelic contribution from ILC 3279

Table 5. 24: Quantitative trait loci detected for days to flowering (DFF), days to maturity (DM), reproductive period (RP) and plant height (PH) at one location in 2006 and two locations in 2007.

Trait ^a	Trial ^b	Linkage group	Interval	Interval length (cM)	Nearest locus to the maximum. LOD peak	Threshold LOD Score*	Maximum LOD score	Additive effects [^]	% Explanation (R ²)
DFF	TH06	LG1	H5A08-TA8	2.5	H5A08	2.2	6.2	1.88	13
	TH06	LG3	TR24-TR26	69.0	TR56	2.2	9.7	2.71	28
	TH06	LG4	TA132-TAA170	19.0	TA72	2.2	2.2	1.25	5
	BR07	LG6	TA14-STMS15	5.0	TA14	2.2	2.7	1.25	15
DM	TH06	LG1	H5A08-TA8	2.5	TA8	2.3	7.3	3.61	13
	TH06	LG3	TR24-TR26	69.0	TR56	2.3	16.7	7.2	49
	TH07	LG3	TR24-TR26	69.0	TR56	2.2	5.9	0.95	26
	TH06	LG7	STMS25-TS46	14.0	TA21	2.3	2.9	2.31	5
RP	TH06	LG1	H5A08-TA8	2.5	TA8	2.3	3.9	1.83	7
	TH06	LG3	TR24-TR26	69.0	TR56	2.3	12.8	3.80	37
	BR07	LG3	TR24-TR26	69.0	TR56	2.3	3.7	1.48	25
	BR07	LG3	TS29-TR24	18.0	TS29	2.3	4.8	1.54	26
	TH06	LG7	STMS25-TS46	15.0	TA21	2.3	2.9	1.61	6
PH	TH06	LG1	H5A08-TA8	2.5	TA8	2.1	8.5	2.07	21
	TH06	LG3	TA125-TR26	37.0	TR56	2.1	3.4	1.98	20
	TH07	LG6	NCPGR4-TA106	17.0	NCPGR4	2.3	2.8	1.45	14
	TH07	LG8	TA118-TA25	29.0	TA25	2.39	2.4	-1.28	14

^a DFF, number of days from sowing to first flower (when 50% plants of line showed first flower); DM, number of days from sowing to physiological maturity; RP, number of days between flowering and physiological maturity; PH, plant height (cm)

^b TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

* Threshold level to declare a QTL significant was determined by performing 1000 permutation of the data by maintaining the type I error rate of 0.05

[^] Negative values indicated allelic contribution from ILC 3279

Table 5. 25: Quantitative trait loci detected for stomatal conductance (SC), canopy temperature differential (Tc-Ta) and chlorophyll fluorescence parameters at one location in 2006 and two locations in 2007.

Trait ^a	Date	Trial ^b	Linkage group	Interval	Interval length (cM)	Nearest locus to the maximum LOD peak	Threshold LOD Score*	Maximum LOD score	Additive effects [^]	% Explanation (R ²)
SC	May 11	TH06	LG7	STMS25-TS46	14.0	TA28	2.2	2.6	29.2	9
Tc-Ta	April 25	BR07	LG1	H5A08-H3D05	2.2	H5A08	2.4	2.7	-0.22	13
	May 10	TH06	LG3	TR24-TR56	40.0	TA125	2.4	3.5	0.47	18
	May 25	TH06	LG6	STMS2-TR44	20.0	TA80	2.2	2.6	0.17	8

^a SC, Stomatal Conductance (g_s, mmol m⁻² s⁻¹); Tc-Ta, Canopy temperature differential (°C)

^b TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

* Threshold level to declare a QTL significant was determined by performing 1000 permutation of the data by maintaining the type I error rate of 0.05

[^] Negative values indicated allelic contribution from ILC 3279

height (cm) and canopy temperature differential. The region on LG2 between TR19 and TR13 (3.0 cM) was only associated with drought tolerance score. Three regions on LG3 were associated significantly with most drought related traits. The region between TA125 and TR26 (37.0 cM) was associated with grain yield under drought, HI and plant height, while an extended region between TR24 and TR26 (69.0 cM) was associated with DTS, days to flowering, days to maturity, reproductive period and canopy temperature differential. A third region on LG3, between TS29 and TR24 (18.0 cM), was only associated with reproductive period. There were two distinct regions on LG4, one between TA46 and TA132 (32.4 cM) which was associated with HI and DTS while other region, between TA132 and TAA170 (19.0 cM), was associated only with days to flowering. Similarly, four regions were associated with traits including DTS, days to flower, plant height and canopy temperature differential. One genomic region on LG7, between STMS25 and TS46 (15 cM), was associated with DTS, days to maturity, reproductive period and stomatal conductance, while the region on LG8 between markers TA118 and TA25 (29.0 cM) was associated with HI, DTS and plant height.

The majority of these QTLs were identified in the TH06 trial but not the other two trials. QTLs identified in more than one trial were for grain yield on LG1, HI on LG1, days to maturity on LG3 and reproductive period on LG3 (Table 5.23 – 5.25). There were also some unique QTLs identified in the BR07 trial which was the driest low yielding site. These QTLs were on LG8 for HI, on LG2 and LG8 for drought tolerance score, on LG6 for days to flowering, on LG3 for reproductive period and on LG1 for canopy temperature differential.

5.4.13.1 QTL associated with yield under drought, HI and drought tolerance score

Two QTLs for grain yield under drought were detected on LG1 and LG3, five QTLs for harvest index (HI) on LG1, LG3, LG4 and LG8 and seven QTLs for drought tolerance score (DTS) on LG1, LG2, LG3, LG4, LG6, LG7 and LG8 (Table 5.23). In several cases, markers associated with yield or HI were also associated with DTS and / or phenological traits (Fig. 5.13; Tables 5.23-5.25). This indicates the likely pleiotropic effects of a single genomic region on multiple traits. The QTL on LG1 was associated with both grain yield and HI at the Tel Hadya site in both years of trials (TH06 & TH07). This region explained up to 11% of phenotypic variability for grain yield under drought and 13% for harvest index. This region also had impact on DTS (LOD score = 5.3, % explanation = 10), which is a visual score based on plant vigour, number of pods, etc. Another important QTL was on LG3 which had an effect on many drought tolerance traits. This QTL explained 16% of the phenotypic variability for grain yield, 29% of the variability for HI and 35% for DTS. One relatively weak QTL was identified on LG4 between TA46 and TA132 which showed association with HI (LOD score = 2.5, % explanation = 10) and DTS (LOD score = 2.4, % explanation = 9).

The QTL identified on LG8 was specifically expressed at the Breda site (BR07) for both HI with a LOD score of 2.7 (% explanation = 23) and DTS with a LOD score of 5.0 (% explanation = 33). Two minor QTLs on LG6 and LG7 were also associated with DTS explaining 14% and 5% of the total phenotypic variability, respectively. The QTL found on LG2 was only associated with DTS. Because DTS is a visual score based on many attributes, this region may also control some other drought related traits not measured in this study.

5.4.13.2 QTL for phenology and plant height

Four QTLs were identified for days to flowering on LG1, LG3, LG4 and LG6. QTL on LG1 and LG3 were also associated with days to maturity, reproductive period and plant height which indicated their possible pleiotropic nature. The QTL on LG1 explained 13% of the total phenotypic variability for days to flowering with a LOD score of 6.2. This QTL also explained 13% of the phenotypic variability for days to maturity (LOD score = 7.3), 7% of the phenotypic variability for reproductive period (LOD score = 3.9) and 21% of the total phenotypic variability for plant height (LOD score = 8.5). Similarly, a QTL on LG3 explained 28% of the total phenotypic variability for days to flowering with a LOD score of 9.7. This QTL was also associated with days to maturity in two trials (TH06 & TH07) and reproductive period in two trials (TH06 & BR07). This QTL explained 49% and 26% of the total variability with LOD scores of 16.7 and 5.9 in TH06 and TH07 trials, respectively. Furthermore, this QTL also showed strong association with reproductive period explaining 37% and 25% of the total variability with a LOD score of 12.8 and 3.7 in TH06 and BR07 trials, respectively. This QTL was also associated with plant height explaining 20% of the total phenotypic variability with a LOD score of 3.4 in the TH06 trial. Two other QTLs on LG4 and LG6 were only associated with days to flowering explaining 5% and 15% of the phenotypic variability with 2.2 and 2.7 LOD scores, respectively. The QTL on LG4 was only expressed in the TH06 trial while the QTL on LG6 was only expressed in the BR07 trial. The QTL on LG7 was associated with days to maturity and reproductive period but explained only 5-6% of the total phenotypic variability in both trials. Two QTLs, on LG6 and LG8, were

unique for plant height explaining a total of 28% of the phenotypic variability. These two QTLs were only detected in the TH07 trial.

5.4.13.3 QTL for stomatal conductance and canopy temperature differential

The QTL identified for g_s was on LG7 between STMS25 and TS46 and explained 9% of the total phenotypic variability. Among the three QTLs identified for canopy temperature differential, the QTL on LG3 (LOD score = 3.5) explained 18% of the total phenotypic variability, while QTL on LG1 and LG6 explained 13% and 8% of the total phenotypic variability, respectively. These three QTLs collectively explained 39% of the total phenotypic variability for canopy temperature differential.

5.5 Discussion

The use of molecular markers to study drought tolerance in a set of recombinant inbred lines is a powerful approach for dissecting the genetics of this complex trait. The use of common SSR markers has enabled alignment of the different maps. The level of polymorphism found in this study (42%) was similar to that found by Tar'an *et al.* (2007). Udupa and Baum (2003) and Cho *et al.* (2004) demonstrated that the frequencies of SSR polymorphism between two *C. arietinum* parents were in the range of 30% to 50%. A higher polymorphism frequency (77%) was found between parents of a cross between *C. arietinum* and *C. reticulatum* (Tekeoglu *et al.*, 2002). The issue of low polymorphism might be resolved by combining SSRs with new types of markers, such as those based on single nucleotide polymorphisms (Tar'an *et al.*, 2007).

Chickpea (*Cicer arietinum* L.) has a diploid set of chromosomes ($2n=2x=16$) with an estimated genome size of 740 Mb (Arumuganathan and Earle, 1991). Several linkage maps have been developed for chickpea (Winter *et al.*, 2000; Tekeoglu *et al.*, 2002; Flandez-Galvez *et al.*, 2003; Udupa and Baum, 2003; Cho *et al.*, 2004; Tar'an *et al.*, 2007). Winter *et al.* (2000) mapped a total of 354 markers including 118 SSR types while Tar'an *et al.* (2007) mapped 144 SSR markers. The present study generated eight linkage groups with 19% of the markers showing segregation distortion, which is similar to the segregation distortion ratio (20.4%) obtained by Flandez-Galvez *et al.* (2003) for chickpea SSR markers on an intraspecific chickpea linkage map. Interestingly, the majority of the distorted markers were on LG5. Recombination for the majority of the markers on LG5 was distorted in favour of the genome of the drought susceptible parent, ILC 3279. Winter *et al.* (1999) reported a general trend of distorted segregation in a RIL population from a wide cross and noted that distorted frequencies are more pronounced in RILs than in F_2 population (for this comparison, see Tanksley *et al.*, 1992; Paran *et al.*, 1995). Reasonable genomic synteny was found between the current intraspecific kabuli chickpea map and earlier maps (Winter *et al.*, 2000; Tar'an *et al.*, 2007), which encourages the use of SSR markers and the possibility of integration of different maps through common markers. Some of the linkage groups have short span, e.g., LG1, which could be due to the unavailability of polymorphic markers, potentially due to the genetic relatedness of the parents used to produce the RILs. Hence, attention should be given in the future to identify more polymorphic markers to extend the length of these linkage groups.

The primary objective of the current study was to map genetic loci associated with traits related to drought tolerance in kabuli chickpea. In this study, 13 genomic regions were shown to be associated with drought tolerance traits. Some of these regions showed association with multiple traits, likely because of correlated responses of these traits. Plant phenology strongly interacts with the expression of drought tolerance and yield potential (Ludlow and Muchow, 1990). In this study, days to flowering and days to maturity were strongly correlated with grain yield under drought, harvest index (HI) and drought tolerance score (DTS). This was also evident from QTL analysis where various genomic regions were associated with both phenology and drought tolerance. For example, genomic regions on LG1 and LG3 associated with grain yield under drought, also showed strong association with HI, DTS, days to flowering, days to maturity, reproductive period, plant height and canopy temperature differential. Some of the QTLs on LG3 showed very high LOD score along with high % explanation for important traits under drought (eg. days to flowering (LOD: 9.7, % explanation: 28), days to maturity (LOD: 16.7, % explanation: 49), drought tolerance score (LOD: 12.9, % explanation: 35%), harvest index (LOD: 9.9, % explanation: 29%)), thus LG3 seems to have important regions in relation to drought tolerance. Tar'an *et al.* (2007) also detected a QTL for aschochyta blight resistance on LG3 between TA64 and TS19.

Most of the quantitative trait loci (QTLs) reported here were identified during TH06 as compared to other trials (TH07 and BR07). Possible reasons could be the missing RILs during the TH07 and BR07 which were included in the TH06 trial. Another reason could be the difference in environmental conditions among the three trials.

So far, two studies have reported QTL for days to flowering in chickpea. Cho *et al.* (2002) reported one QTL for days to flowering on LG3, whereas Lichtenzveig *et al.* (2006) reported three QTLs for days to flowering on LG1, LG2 and LG8. One QTL for days to flowering found in this study on LG1 (LOD = 6.2, % explanation = 13) and by Lichtenzveig *et al.* (2006) on LG1 (LOD = 10, % explanation = 60) could be the same gene or set of genes, although it is hard to match both regions due to lack of common markers. Another major QTL found in this study for days to flowering was on LG3. Cho *et al.* (2002) also reported a QTL for days to flowering on LG3 however, it may not have been LG3, but rather LG8, as suggested by the presence of common markers between this linkage group, LG8 of Tar'an *et al.* (2007) and the current map.

Later, Lichtenzveig *et al.* (2006) also found a QTL for days to flowering on LG8 with a LOD score of 3.5. Hence, LG3 and LG8 are also strong candidate linkage groups having QTLs controlling flowering. Two additional QTLs with smaller effects detected on LG4 and LG6 are reported for the first time in this study. The QTL on LG6 was expressed only at the dry BR07 site. Total phenotypic variability explained by these four QTLs identified in this study is 61%. Inheritance studies reported by Anbessa *et al.*, (2006) also showed that time to flowering is determined by two major genes plus polygenes.

This study revealed that higher stomatal conductance (g_s) and cooler canopies are associated with higher grain yield under drought stress in chickpea. This g_s -yield relationship agrees with studies on wheat (Condon *et al.*, 1987; Sayre *et al.*, 1995; Fischer *et al.*, 1998) and barley (González *et al.*, 1999). The use of canopy temperature as an indirect selection criterion under drought conditions has been documented in wheat

(Blum *et al.*, 1982; Pinter *et al.*, 1990; Araghi and Assad, 1998), barley (González *et al.*, 1999) and pearl millet (Singh and Kanemasu, 1983), due to its convenient and quick measurement by hand-held infrared thermometers. These two traits have the potential to be used in screening chickpea drought tolerant genotypes. Genotypes which can maintain a higher level of g_s during the reproductive period and keep their canopies cooler than air temperature under a cycle of drought can produce better yields. This study revealed that some chickpea genotypes were able to maintain higher g_s ($>300 \text{ mmol m}^{-2}\text{s}^{-1}$) and cooler canopies ($T_c - T_a < -1^\circ\text{C}$) as compared to other genotypes which have lower g_s ($<200 \text{ mmol m}^{-2}\text{s}^{-1}$) and warmer canopies ($T_c - T_a > -1^\circ\text{C}$). Conventional selection procedures for the selection of genotypes on the basis of these two traits is difficult because of the extremely low or negligible heritability estimates ($<9\%$ for g_s ; $<17\%$ for $T_c - T_a$), mainly because of large environmental variances. A possible solution could be the identification of molecular markers which are environmentally insensitive and which could be used for efficient selection. In this study, one QTL was detected for g_s on LG7 explaining 9% of the total variability, while three QTLs were detected for canopy temperature differential on LG1, LG3 and LG6 explaining together 39% of the total phenotypic variability. The genomic regions on LG1, LG3, LG6 and LG7 were also associated with traits of higher productivity under drought and drought tolerance score (DTS). Hence, these genomic regions appeared to be important for enhanced drought tolerance in chickpea.

Berger *et al.* (2006) confirmed the importance of high HI and drought escape in chickpea under natural drought stress through early flowering, podding and maturity. This finding agrees with the role of earliness as a drought escape mechanism in other crops including wheat and barley under Mediterranean environments (Acevedo *et al.*,

1991; González *et al.*, 1999). The current study revealed that improved grain yield under drought condition was associated with higher HI, early flowering and early maturity. Plants having higher HI have better partitioning ability of photosynthetic assimilates into grain development under drought stress conditions. This ability should help the crop in improving its stability of performance under different climatic conditions and reducing volatility compared to selection based solely on grain yield.

Differences for chlorophyll fluorescence parameters were not detected between drought tolerant and susceptible RILs in this population. There could be two explanations for this situation. Firstly, this was likely due to the minimal differences in these parameters between the parental genotypes. Further studies of chlorophyll fluorescence parameters on a population with more diverse parents for fluorescence parameters might produce more informative results. Secondly, in this study, chlorophyll fluorescence parameters were measured on dark adapted leaves, which gave the maximum quantum efficiency of PSII (F_v/F_m). Drought stress imposed minimal or no impact on maximum efficiency of PSII. With increasing water loss, inhibition of photosynthetic metabolism can occur and result in a decline in photosynthetic potential and a further decrease in the CO_2 assimilation rate (Lawlor and Cornic, 2002). Ultimately, drought will decrease the rate of utilization of ATP and NADPH and consequently, decrease the operating efficiency of PSII ($F'q/F'm$) (Fracheboud and Leipner, 2003). Monitoring of $F'q/F'm$ may prove useful for rapid screening of tolerance to severe water stress. Operating efficiency of PSII ($F'q/F'm$) can be derived by measuring chlorophyll fluorescence parameters (F' , $F'm$, $F'q$) on light adapted leaves (Baker and Rosenqvist, 2004).

This study provided insight into the genetic control of various traits related to productivity of chickpea under drought conditions. The addition of further markers to increase the density of the map will be helpful to refine these findings. Further study of additional breeding populations is necessary to validate the presence of QTL for the improvement of drought tolerance of chickpea by marker-assisted selection.

6. General discussion and conclusions

Conventional breeding for drought tolerance is primarily based on selection for yield and its components under a given drought stress environment. Because of the variability in drought pattern from year to year, trait-based selection could have an advantage. Trait-based breeding, however, requires trait dissection into components. Substantial efforts have targeted the manipulation of morpho-physiological traits influencing drought resistance through escape, avoidance and/or tolerance mechanisms (Ludlow and Mochow, 1990; Blum, 1996; Turner *et al.*, 2001).

Breeding for drought tolerance is not simple. Under a particular environment, some physiological or metabolic processes can be modified through breeding, either as single traits or as a combination of traits. Traits including modification of the root system, stomatal control, and leaf area, as well as matching plant phenology with the environment, could help in improving productivity under drought stress conditions. Moreover, identification of QTLs for the key traits responsible for improved productivity under drought could be helpful in accelerating the process of pyramiding of favourable alleles into adapted genotypes for better production. This requires integration of knowledge from plant physiology and biotechnology into plant breeding. Understanding plant response to water stress for key drought stress traits and screening of mapping populations for these traits for QTL identification are of prime importance for future drought stress breeding.

6.1 Trait association with drought tolerance

This study revealed that improved grain yield under drought conditions in Mediterranean environments was associated with higher harvest index, early flowering and early maturity. Drought tolerance score (DTS) was associated with various important traits including grain yield, harvest index, grain number, grain weight, days to flowering and maturity. Ludlow and Muchow (1990) reported a strong interaction of plant phenology with the expression of drought tolerance and yield potential. Anbessa *et al.*, (2007) reported a positive effect of double podding and early flowering on reducing the days to crop maturity in chickpea. This study also supported early flowering, early maturity and high harvest index being beneficial traits under progressive drought stress in chickpea.

This study also concluded that chickpea genotypes differed in their capacity to respond to water deficits (chapter 3) and can be exploited for developing drought tolerant genotypes. In contrast to earlier reports (Ludlow and Muchow, 1990; Saxena and Johansen, 1990; Turner *et al.*, 2001) suggesting a positive role of deeper and/or larger root system in higher grain yield in crops, the group of four genotypes having the largest root system under greenhouse conditions (ILC 3279, ILC 10606, ILC 9955, and Amit) also had lower grain yield and harvest index under three moisture stress treatments, as compared to the other four genotypes (ILC 588, ILC 3182, ICCV 2, and CDC Chico). One should also consider the efficiency of the root system vs. the size of the root system, since in this research, large root systems were offset by a low harvest index, presumably due to the lack of assimilate available for grain growth. Although root traits were not tested on the RIL populations under field conditions, it would be interesting to investigate

the rooting behaviour of the two groups of RILs contrasting in grain yield, harvest index and drought tolerance scores.

This study also reported a significant association of stomatal conductance (g_s) with each of HI, grain yield, drought susceptibility index and drought tolerance score (DTS). Stomatal conductance could be used to assess plant stress due to drought. Values of g_s less than $250 \text{ mmol m}^{-2}\text{s}^{-1}$ during flowering indicated drought stress (chapter 4). A higher degree of plant stress due to drought was shown by increased stomatal closure at midday ($g_s < 150 \text{ mmol m}^{-2}\text{s}^{-1}$). The study of 157 RILs under natural drought stress during 2005-07 revealed that the RILs which had better grain yield under drought (Group A) had higher g_s than the RILs that had lower grain yield (Group B). Group A had g_s values of $390 \text{ mmol m}^{-2}\text{s}^{-1}$ one week before flowering while Group B had $330 \text{ mmol m}^{-2}\text{s}^{-1}$. Stomatal conductance increased at flowering and then sharply decreased later in reproductive period due to severe drought stress.

Analysis of sub-soil water content during the experiment period revealed a severe reduction of moisture in the upper 45 cm soil depth (chapter 5). Under severe drought stress, Group A was still able to transpire and had higher g_s ($170 \text{ mmol m}^{-2}\text{s}^{-1}$) compared to Group B ($110 \text{ mmol m}^{-2}\text{s}^{-1}$) at 10 weeks after sowing. Group A was able to maintain higher g_s during the reproductive period and were also able to produce greater yield. These findings were also supported by canopy temperature differential ($T_c - T_a$) measurements as Group A was also able to maintain lower canopy temperature than the Group B, which indicated the ability of these plants to maintain adequate transpiration and maintain a cooler canopy. The overall results indicated that g_s and canopy temperature could be used to assess plant drought stress and to screen for drought tolerant

genotypes. Use of these two traits for selection under field conditions is not simple, because both traits are environmentally sensitive (see chapter 4 and 5) and require extra care. Further, the heritability estimates of these traits are low due to their larger environmental variances, making the selection process difficult. A possible solution is the identification of QTL associated with g_s and canopy temperature differential for use in future breeding for drought tolerance.

6.2 Mapping QTL associated with drought tolerance

Drought stress tolerance is a complex and quantitatively inherited trait, controlled by several genetic loci. QTL analysis in genetically fixed population e.g. recombinant inbred lines, facilitates the dissection of the genetic basis of drought tolerance. Successful marker identification would facilitate integration of MAS procedures in breeding programs enabling the pyramiding of favourable alleles and target loci. The development of a dense linkage map for chickpea containing a large number of molecular markers is required. The map produced in this study containing 52 SSR markers spanned 335 cM of the chickpea genome at an average density of 6.4 cM. Comparatively larger maps have been reported in earlier studies (see Discussion, chapter 5) where they combined different marker types including AFLP, SSR etc. This emphasizes the need to incorporate more common type of markers in the map. The use of common SSR markers will enabled the alignment and integration of different maps (for example, Winter *et al.*, 2000; Tar'an *et al.*, 2007) and allows for the possibility for the development of a consensus map of chickpea.

The primary objective of the current study was to map genetic loci associated with traits related to drought tolerance in kabuli chickpea. In this study, 13 genomic

regions were shown to be associated with drought tolerance traits. The majority of these regions were detected in the TH06 trial as compared to the other two trials, likely due to the missing RILs in the other two trials. Some of these genomic regions showed pleiotropic effect on multiple traits. This was also supported by the analysis of phenotypic data where these traits were found to be correlated. For example, early flowering and maturity had strong association with higher grain yield. Higher grain yield was also associated with high harvest index. Drought tolerance score (DTS) was associated with various important traits including grain yield, harvest index, grain number, grain weight, days to flowering and maturity. This study also reported a significant association of stomatal conductance (g_s) with each of HI, grain yield, drought susceptibility index and drought tolerance score (DTS).

Thirteen genomic regions identified in this study were associated with various important drought related traits including grain yield under drought, HI, DTS, days to flowering, days to maturity, stomatal conductance and canopy temperature differential. Two QTLs for grain yield under drought were detected on LG1 and LG3 while five QTLs were detected for HI explaining 86% of the total phenotypic variability for the trait. Four QTLs were detected for days to flowering explained 61% of the total phenotypic variability. Inheritance studies showed that time to flowering is determined by two major genes plus polygenes (Anbessa *et al.*, 2006). QTLs associated with other related traits are also reported. This study also identified one QTL on LG7 for g_s and three QTLs on LG1, LG3 and LG6 associated with canopy temperature differential. These findings will help in the further development and future use of MAS in incorporating drought tolerance into chickpea.

6.3 Implications for breeding for drought tolerance

The main strategy of crop breeders and physiologists in developing stress tolerant cultivars is to reduce the volatile nature of productivity due to stresses. Drought tolerance is a complex mechanism and can be achieved with the accumulation of favourable genes for traits important for higher productivity under drought stress. Various traits related to escape, avoidance or tolerance mechanisms can be considered depending upon the target environment. A deeper root system could be a useful trait for chickpea cultivars for regions where the crop generally grows on stored moisture and progressive drought stress conditions. On the other hand, a comparatively restricted root system could be better suited to regions with short growing seasons such as Western Canada where crop growth termination is required prior to fall frost. High harvest index and drought escape through early flowering and early maturity are also important attributes in drought stressed environments. Higher stomatal conductance (g_s) and cooler canopies were found to be associated with higher grain yield under drought stress conditions. Higher g_s and cooler canopies are characteristic of plants which are able to obtain and utilize moisture in photosynthesis. These kinds of plants can also be termed as ‘water spenders’, as opposed to ‘water savers’ where plants try to utilize less water after receiving a drought signal to save water for later growth. Water spenders may exhaust water rapidly as compared to water savers. This research showed that water spenders used more water as shown by their higher g_s and lower canopy temperature, but also produced greater grain yield.

Hence, cultivars can be developed or selected on the basis of higher g_s and cooler canopies under drought stress conditions. Various QTLs identified in this research for traits related to higher productivity under drought stress could have important

implications on accelerating the process of pyramiding of favourable genes into adapted genotypes and on future marker-assisted breeding for drought prone areas.

This research was conducted under terminal drought stress where the crop grows mainly on stored soil moisture, or where early crop growth occurs under good moisture conditions and later crop growth occurs under severe drought stress and high temperature. Hence, the scope of this research is limited to such areas, although some generalities can be drawn for other areas as well.

6.3 Future research

Future research work arising from this thesis should be directed towards the following areas:

- a) Increasing the coverage of genome and marker density of the linkage map developed in this study would improve its usefulness by increasing the likelihood that markers will be tightly linked to genes of interest. This will also facilitate the alignment of different chickpea genome maps and ultimately will help in the development of a chickpea consensus map.
- b) Recombinant inbred lines used in this study were only tested in two environments in Syria. These RILs can also be tested in additional environments to study if QTLs detected in this study are also important in other environments.
- c) A second population of recombinant inbred lines developed from ILC 3182 x ILC 3279 was phenotyped for various traits (Appendix I) under two environments in Syria during 2006 and 2007. This population could also be used for genotyping and QTL detection for drought tolerance which will strengthen the QTLs detected in this study.

d) This study provided evidence about the association of g_s and canopy temperature with higher productivity under drought stress. This study also suggested the role of phenology in avoiding drought stress under a given environment. Genotypes selected on the basis of these traits, if tested in multiple drought environments, should provide better stability of grain yield.

e) In this study, chlorophyll fluorescence parameters were measured on dark adapted leaves, which gave the maximum quantum efficiency of PSII (F_v/F_m). Maximum quantum efficiency of PSII could not give clear indication of its role in assessing the plant stress under drought in chickpea. Alternatively, the operating efficiency of PSII ($F'q/F'm$) might have role in assessing plant stress under drought. Monitoring of $F'q/F'm$ may prove useful for rapid screening of tolerance to severe water stress.

7. References

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Appendix I.

List of parental genotypes and RILs included in QTL analysis

DNA code	Genotype	DNA code	Genotype	DNA code	Genotype
1	ILC3279 (P)	45	DR1-199	89	DR1-17
2	ILC588 (P)	46	DR1-201	90	DR1-27-A-1
3	DR1-10	47	DR1-209-A-1	91	DR1-27-B-1
4	DR1-19	48	DR1-209-B-1	92	DR1-45-A-1
5	DR1-28	49	DR1-211	93	DR1-47
6	DR1-29	50	DR1-212	94	DR1-74-1
7	DR1-37	51	DR1-217	95	DR1-76-A-1
8	DR1-43	52	DR1-218	96	DR1-81-A-1
9	DR1-49	53	DR1-219	97	DR1-81-B-1
10	DR1-50	54	DR1-220	98	DR1-86-A-1
11	DR1-65	55	DR1-222	99	DR1-106-A-1
12	DR1-67	56	DR1-223	100	DR1-106-B-1
13	DR1-68	57	DR1-225	101	DR1-108-A
14	DR1-69	58	DR1-226	102	DR1-118
15	DR1-70	59	DR1-229	103	DR1-119-A-1
16	DR1-71	60	DR1-230	104	DR1-119-B-1
17	DR1-73	61	DR1-232	105	DR1-124-A-1
18	DR1-78	62	DR1-234	106	DR1-135
19	DR1-79	63	DR1-237	107	DR1-141
20	DR1-85	64	DR1-238	108	DR1-157
21	DR1-88	65	DR1-239	109	DR1-159-A-1
22	DR1-89	66	DR1-241	110	DR1-159-B-1
23	DR1-109	67	DR1-242	111	DR1-176
24	DR1-110	68	DR1-244	112	DR1-181
25	DR1-114	69	DR1-248	113	DR1-184-A-1
26	DR1-115	70	DR1-251	114	DR1-184-B-1
27	DR1-116	71	DR1-252-A-1	115	DR1-213
28	DR1-122	72	DR1-252-B-1	116	DR1-215-A-1
29	DR1-126	73	DR1-253	117	DR1-215-B-1
30	DR1-127	74	DR1-255	118	DR1-228-B-1
31	DR1-140	75	DR1-257-A-1	119	DR1-241-A-1
32	DR1-145	76	DR1-257-B-1	120	DR1-241-B-1
33	DR1-147	77	DR1-258-A-1	121	DR1-250-A-1
34	DR1-152	78	DR1-265	122	DR1-259-B-1
35	DR1-154	79	DR1-270	123	DR1-262
36	DR1-156	80	DR1-272	124	DR1-266-A-1
37	DR1-167	81	DR1-273	125	DR1-266-B-1
38	DR1-175	82	DR1-277	126	DR1-269
39	DR1-186	83	DR1-278	127	DR1-274
40	DR1-188	84	DR1-279	128	DR1-283-A-1
41	DR1-192	85	DR1-3	129	DR1-283-B-1
42	DR1-194	86	DR1-11	130	DR1-124-B-1
43	DR1-197	87	DR1-14-A-1		
44	DR1-198	88	DR1-14-B-1		

Appendix II.

Screening of kabuli chickpea population of recombinant inbred lines from the cross between ILC 3182 and ILC 3279 for various agronomic traits under a Mediterranean environment.

Summary

Drought is the most important abiotic stress in many chickpea growing regions and occasionally severe drought conditions lead to complete crop failure. The present study was envisaged to characterize a chickpea mapping population of a cross between drought tolerant and susceptible genotypes (ILC 3182 and ILC 3279, respectively) developed through single seed descent method. The population consisting of 121 F_{6:7-8} recombinant inbred lines (RILs) was studied under natural drought conditions in the field at Tel Hadya, Syria in 2006 (TH06) and 2007 (TH07) and at Breda, Syria in 2007 (BR07) for various agronomic traits. Drought tolerance score (DTS) was correlated with grain yield (g plant⁻¹) at TH06 (-0.66, P<0.01), TH07 (-0.49, P<0.01) and BR07 (-0.56, P<0.01). DTS was also correlated significantly with HI, number of grains per plant and number of pods per plant in all three trials. A positive correlation was observed between shoot dry weight (SDW) and grain yield at TH06 (0.74, P<0.01), TH07 (0.85, P<0.01) and BR07 (0.64, P<0.01). Harvest index was also correlated positively with grain yield at TH06 (0.79, P<0.01), TH07 (0.62, P<0.01) and BR07 (0.79, <0.01). Days to flowering and maturity were negatively correlated with grain yield in all three trials. Results obtained from these studies revealed that high harvest index, early flowering, early maturity, large number of grains and pods per plant and high 100-grain weight were the important attributes contributing to higher grain yield under drought.

Introduction

Chickpea (*Cicer arietinum* L.) is an important legume crop in the Semi-Arid Tropics (SAT) and the West Asia and North Africa (WANA) regions, and is becoming an important legume crop in new regions like Australia and North America. Although chickpea is known for its better drought tolerance than most other cool-season legumes, drought does reduce yields and can even lead to total crop failure. In both Mediterranean and sub-tropical climates, seed filling in chickpea is subject to terminal drought, which limits seed yield (Turner *et al.*, 2001). Many physiological processes associated with crop growth and development are influenced by water deficits (Hsiao, 1973; Boyer and McPherson, 1975; Begg and Turner, 1976; and Turner and Begg, 1978). Early maturing chickpea varieties that escape terminal drought have been developed (Kumar and Abbo, 2001), but early maturity places a ceiling on the potential yield and limits the crop's ability to exploit extended growing periods. Increasing the drought avoidance of the crop should help to stabilize yields at higher levels than possible with escape (Johansen *et al.*, 1997).

In chickpea, the focus of drought resistance research is on the ability to sustain greater biomass production and crop yield under a seasonally increasing water deficit, rather than the physiological aptitude for plant survival under extreme drought shock (Serraj and Sinclair, 2002). This has led to the focus on escape and avoidance strategies such as early maturity (Kumar and Abbo, 2001). In this research, homozygous F_{6:7-8} recombinant inbred lines (RILs) developed at the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria were used to study various drought tolerance indicator parameters. Hence, the specific objective of this study was to measure

the phenotypic expression of agronomic and physiological traits associated with drought tolerance in a set of RILs from an intraspecific chickpea population.

Materials and Methods

Plant Material

An intraspecific cross of kabuli chickpea between ILC 3182 and ILC 3279 was used to develop a RIL population. ILC 3182 is drought tolerant and ILC 3279 is drought susceptible genotypes based on their yield performance under drought conditions (personal communication, R.S. Malhotra, ICARDA, Syria). The cross was advanced through single seed descent method in the field and plastic house at ICARDA. A total of 121 $F_{6:7-8}$ RILs were used for genetic analysis for drought tolerance. Thus, $F_{6:7-8}$ lines along with parental genotypes were evaluated for yield components and drought tolerance during 2006 and 2007 at two locations i.e. Tel Hadya and Breda, Syria.

Site description

The study was conducted under field conditions in three environments i.e. one location (Tel Hadya, Syria) during 2006 and two locations (Tel Hadya and Breda, Syria) during 2007. The 25 year average annual rainfall at Tel Hadya is 350 mm while at Breda is 250 mm. The soil characteristics at the three experiments are summarized in Table 5.1 in chapter 5. Soil analysis was carried out at Soil, Plant and Water Analysis Laboratory, ICARDA, Syria using the hydrometer method and USDA textural classification for soil textural classes. Soil pH was determined in 1:1 soil – water ratio (Jackson, 1958), EC in 1:1 soil – water ratio extract, organic matter (OM) by the Walkley and Black method

(Hesse, 1971), total nitrogen (N) by the Kjeldahl method (Hesse, 1971), available phosphorus (P) by Olsen's method (Olsen *et al.*, 1954) and extractable potassium (K) by using an ammonium acetate extractant and flame photometer. Field capacity and wilting point of the soil were determined by pressure plate extractor (1 and 15 bar). Soil particle size analysis was conducted by the hydrometer method (bouyoucos hydrometer).

Experimental procedure

Each trial consisted of two replicates in an alpha-lattice design. Seeds were sown in one meter row plots with a ten centimeter plant to plant and 45 cm row to row distance. Due to a shortage of seed, some RILs were not included in the trials during 2007 while some new lines were included whose seed was increased during the previous year. The total number of RILs tested in the first trial at Tel Hadya during 2006 (TH06) was 121 while 106 RILs were tested during 2007 at Tel Hadya site (TH07) and 80 at Breda site (BR07). Parental genotypes were included in all trials. Trail TH06 was planted on March 22 and harvested on June 28, TH07 was planted at Tel Hadya on March 11 and harvested on June 18, and BR07 was planted on January 4 and harvested on June 09. Seeds were treated with fungicides carboxin, thiram, attapulgate clay (Vitavax) at the rate of 3 g kg⁻¹ for the control of *Rhizoctonia solani*, *Helminthosporium*, *Fusarium* and *Pythium* species. The fungicide chlorothalonil (Bravo) was sprayed at the rate of two liters per hectare (720 g/L chlorothalonil) during the vegetative period for the control of *Aschochyta*.

Measurements for various traits

Measurement of yield and yield related traits

The five middle plants from each row were harvested individually for subsequent measurements. All the measurements were made on an individual plant basis and the means of five plants were used for analysis for yield and related traits. At maturity, plants were harvested by cutting at ground level and placing each plant in separate bags. Plants were oven-dried at 45°C for 48 hours before weighing. Traits measured were grain yield (g plant^{-1}), above ground biomass (g plant^{-1}), number of grains per plant, number of pods per plant, 100-grain weight (g plant^{-1}). Harvest index (HI) was calculated according to the formula:

$$\text{HI} = \text{Grain weight} / \text{Total above ground dry weight}$$

Measurement of morphological traits

Data were collected on days from sowing to flowering by calculating the difference of days from date of sowing to the date when 50% of the plants in a line showed the first fully open flower. Days from sowing to physiological maturity were recorded by calculating the difference of days from date of sowing to the date when 90% of the plants had turned colour. The reproductive growth period was calculated as the days between the start of flowering and physiological maturity. Plant height (cm) was measured just prior to physiological maturity by taking four readings on each line excluding border plants and averaging before analysis.

Measurement of drought tolerance score (DTS)

Drought tolerance score (DTS) (Singh *et al.*, 1997) was used to screen large number of lines in the field under drought conditions. The rating was done at the late pod-filling stage, when the crop was approaching maturity. A scale from 1 to 9 was used with 1 being drought stress free and 9 being inability to set seed in the prevailing stress conditions. (Please refer to chapter 5 for the description of 1-9 scale).

Measurement of soil moisture

Soil moisture was monitored by neutron probes (Didcot Instrument Co. LTD., Abingdon, Oxon, England) at 9 points scattered throughout the trial only at Tel Hadya during both years. Measurements were taken up to 180 cm depth. The instrument was calibrated before the start of the measurements. Tubes were installed and measurements were carried out at biweekly intervals starting from sowing until harvesting of the experiment. The neutron moisture probes consists of a probe containing a source of fast neutrons that move radially outward from the source, a thermal neutron detector, and the associated electronic equipment to supply power for the detector and display the results (Fig. 5.2 A &B, chapter 5). For detail, readers are referred to chapter 5.

Statistical analysis

Individual trial: Each trial was analyzed using the following linear additive mixed effects model

$$Y_{ijk} = \mu + r_i + b_{ij} + g_k + e_{ijk}$$

where Y_{ijk} is the observation of a trait recorded on an experimental plot under the genotype k in incomplete block j of replicate i , μ is the general mean, r_i the effect of replicate i , b_{ij} the effect of block j within replicate i , g_k the effect of genotype k , and e_{ijk} the experimental error from the plot. The effects of replications and blocks within replications were assumed independent and normally distributed random variables with means zero. The experimental errors (i.e. the plot effects) e_{ijk} were assumed independent and normally distributed with mean zero and variance σ_e^2 , and independent of the other random factors in the model. The variance of genotype effects, g_k , was denoted by σ_g^2 .

A measure of broad-sense heritability of trait Y is the ratio of genetic variance (σ_g^2) to phenotypic variance ($\sigma_g^2 + \sigma_e^2$).

$$h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$$

Multi-environment trials: The data from the multi-environment trials were combined and the above model was modified to incorporate fixed effects due to environments while genotype x environment interaction effects were assumed random with mean zero and variance σ_{ge}^2 . The experimental error variances σ_e^2 were assumed constant over the trials. The heritability of a given trait, on a means basis, from all the trials was estimated as follows:

$$h^2 = \sigma_g^2 / \{ \sigma_g^2 + \sigma_{ge}^2 / L + \sigma_e^2 / (RL) \}$$

where R is the number of replications in each trial and L is the number of locations or environments.

Estimation of the variance components in the above model, and its generalized version for multi-environments, were carried out using residual maximum likelihood

(REML) method provided in the PROC MIXED procedure of SAS 8.02 (SAS Institute, Cary, N.C., USA, 1999). Standard error for heritability was estimated as explained by Singh and Ceccarelli (1995).

Results

Weather

Environmental data regarding precipitation, minimum and maximum temperatures are shown in Fig. 5.3 of chapter 5 for the three experimental sites viz. TH06, TH07 and BR07. Total precipitation received at TH06 (from September 2005 to August 2006) was 270 mm, at TH07 for the same period was 300 mm. Precipitation received at BR07 for the same period was 267 mm. Most of the crop growth period during 2006 was drier as compared to 2007 at Tel Hadya where the crop received intermittent rainfall with high temperature during later growth stages in May. Total precipitation received during the trial period at TH06 was only 25.7 mm and at TH07 was 72.6 mm while at BR07 received about 191 mm during the trial period.

Mean monthly minimum temperature at TH06 ranged from 5°C to 12 °C from March to May and further increased to 20 °C during June. Similarly, mean monthly maximum temperature ranged from 20 °C to over 30 °C from March to May and further increased to 35 °C during June. Mean minimum temperature during April 2007 at TH07 was lower in March as compared to 2006 at the same time but abruptly increased during May to 30 °C. Precipitation received during May 2007 was over 40 mm. Similarly, minimum temperature at BR07 ranged from below 0 °C in January to over 5 °C in April

and increased to 15 °C in May. Mean maximum temperature ranged from 12 °C in January to 22 °C in April and 33 °C in May, 2007.

Site description

The soil at the TH06 trial site contained 64% clay, 28% silt and 8% sand (Table 5.1, chapter 5). The soil at the TH07 trial site contained a similar ratio with 62% clay, 25% silt and 13% sand. The soil at the BR07 site contained 40% clay, 29% silt and 30% sand. Organic matter at the three sites was similar around 1% with pH around 8.5 and EC 0.2 mS cm⁻¹. The three sites were relatively rich in total nitrogen. Soil water contents were monitored by neutron probes up to 180 cm depth during the growing season at TH06 and TH07 (Please see Fig. 5.4 & 5.5 of chapter 5).

Changes in soil moisture content from sowing to harvesting were observed up to 75 cm. Moisture level from 75 cm to 180 cm soil depth remained relatively unchanged during the entire growing season. During 2007, there was intermittent rainfall, hence frequent Changes in the soil moisture content in the upper layers can be seen in Fig. 5.5 (chapter 5). In contrast, relatively normal rainfall patterns occurred in 2006 resulting in increased soil dryness from sowing towards harvesting with depleting moisture contents in upper layers.

Yield and yield components

A comparison of mean grain yield (g plant⁻¹) over all the genotypes at the three environments is given in Fig. 1. Mean grain yield was higher at Tel Hadya as compared to Breda. At Tel Hadya, grain yield was higher during 2007 than 2006. Frequency

distribution for grain yield per plant for the three environments is presented in Fig. 2. The drought susceptible parent ILC 3279 had lower mean grain yield than the population mean, while the drought tolerant parent ILC 3182 had higher mean grain yield than the population mean in all three experiments. The means, minimum and maximum along with standard deviation for parental genotypes and RILs for yield and yield components is provided in Table 1. The parents differed significantly for grain yield plant^{-1} under drought and for yield components in all three trials. The difference among RILs was also significant for grain yield and yield components.

The lowest range from minimum to maximum grain yield per plant was obtained during TH06 (0.2 – 4.8 g plant^{-1}), while the highest range of grain yield was obtained during TH07 (2 – 8 g plant^{-1}). Regarding shoot dry weight, the difference between the two parents was maximum during TH06 but minimum during TH07 and BR07. The lowest range of 100-grain weight was obtained during BR07 (12 – 28 g), while the highest range was obtained during TH07 (15 – 31 g). The number of grains per plant ranged from 2 to 24 during TH06 while 11 to 37 during TH07. Similarly, the number of pods per plant ranged from one to 23 during TH06 while 12 to 38 during TH07.

Phenology

The mean values and other relevant statistical parameters for traits including number of days from sowing to flowering (DFF), days to physiological maturity (DM), reproductive period (RP) and final plant height (cm) are presented in Table 2. Frequency distributions for days to flowering and maturity for the three environments are presented in Fig. 3 & 4. The parents differed significantly in DFF, DM and plant height in all three

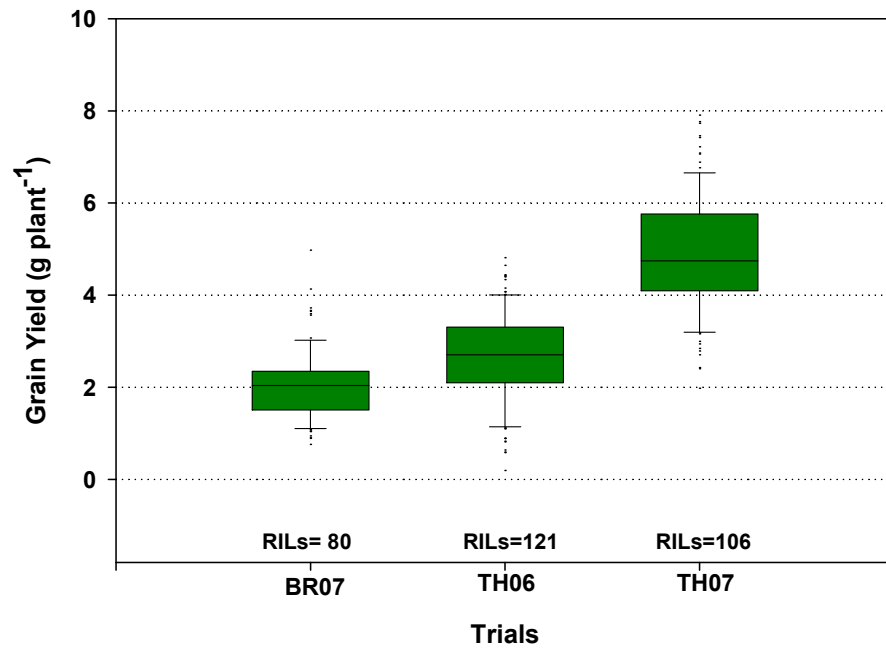


Fig. 1: Box and whisker plot showing mean and variance of RILs of a population of ILC 3182 x ILC 3279 for grain yield (g plant^{-1}) at Tel Hadya, Syria during 2006 and 2007 and Breda, Syria during 2007.

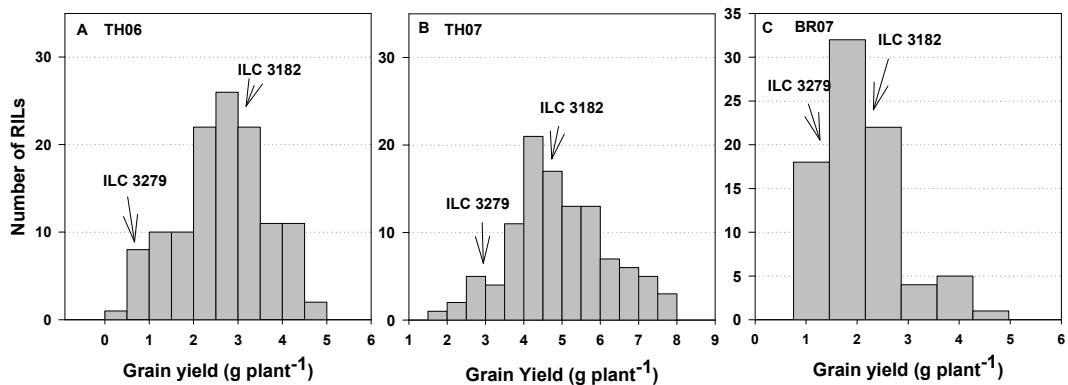


Fig. 2: Frequency distribution of RILs of a cross of ILC 3182 x ILC 3279 for grain yield (g plant^{-1}) for trials conducted at Tel Hadya, Syria during 2006 and 2007 and at Breda, Syria during 2007. Arrows shows the position of both parents of the population in the distribution.

Table 1: Means, standard error and range of the RILs of the cross ILC 3182 x ILC 3279 as well as means for parental genotypes for various agronomic traits under field drought stress conditions during 2006 and 2007.

Trait ^a	Trial ^b	# RILs	Parents		RILs			
			ILC 3182	ILC 3279	Min ^c	Max	Mean	Se(+/-)
Yield	TH06	121	3.12	0.19	0.19	4.81	2.65	0.09
	TH07	106	4.29	3.20	1.97	7.91	4.92	0.12
	BR07	80	1.87	1.03	0.76	4.97	2.06	0.09
SDW	TH06	121	6.70	3.39	3.39	12.61	6.88	0.15
	TH07	106	8.92	9.13	6.07	17.65	11.03	0.21
	BR07	80	8.80	10.16	6.81	17.36	11.60	0.26
100-GW	TH06	121	25.1	13.5	13.5	29.9	22.5	0.3
	TH07	106	26.7	23.0	14.8	30.5	22.5	0.3
	BR07	80	26.8	19.9	11.8	28.0	20.4	0.4
Grain P ⁻¹	TH06	121	12.4	1.6	1.6	23.8	11.8	0.4
	TH07	106	16.0	14.0	10.8	37.3	21.9	0.5
	BR07	80	7.1	4.9	4.4	20.2	10.0	0.4
Pods P ⁻¹	TH06	121	11.9	1.2	1.2	23.3	11.7	0.4
	TH07	106	16.7	14.2	11.8	38.4	22.7	0.5
	BR07	80	10.4	9.3	7.8	27.9	14.3	0.4
Grain Pod ⁻¹	TH06	121	1.1	1.2	0.8	1.4	1.0	0.01
	TH07	106	1.0	1.0	0.6	1.2	1.0	0.01
	BR07	80	0.7	0.5	0.5	1.1	0.7	0.01

^a Yield, Grain yield (g plant⁻¹); SDW, shoot dry weight (g plant⁻¹); 100-GW, hundred grain weight (g); Grain P⁻¹, number of grains per plant; Pods P⁻¹, number of pods per plant; Grain Pod⁻¹, number of grains per pod

^b TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

^c Min, minimum, Max, maximum, se(+/-), standard error (+/-), SD, standard deviation

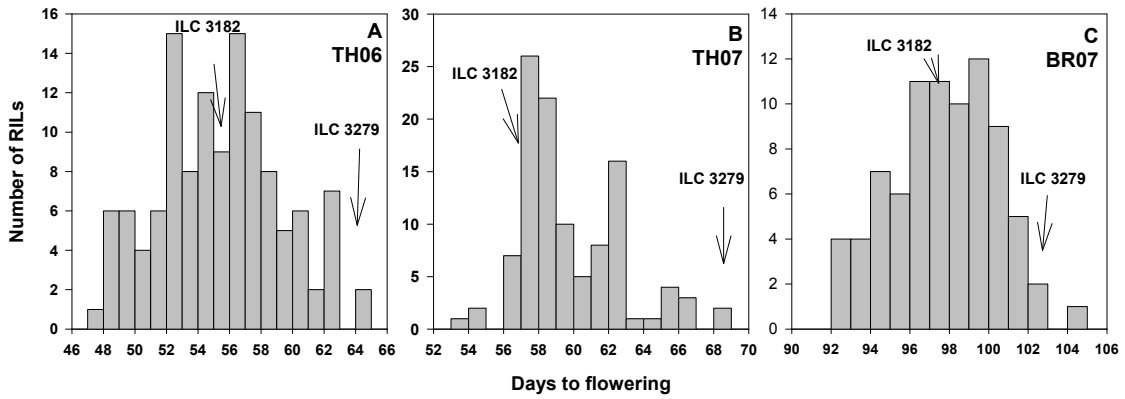


Fig. 3: Frequency distribution of RILs of a cross of ILC 3182 x ILC 3279 for days to flowering for trials conducted at Tel Hadya, Syria during 2006 and 2007 and at Breda, Syria during 2007. Arrows shows the position of both parents of the population in the distribution.

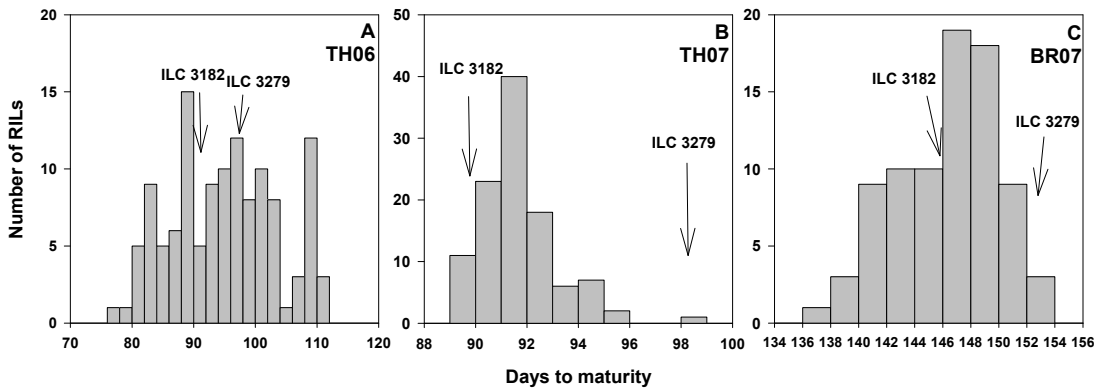


Fig. 4: Frequency distribution of RILs of a cross of ILC 3182 x ILC 3279 for days to maturity for trials conducted at Tel Hadya, Syria during 2006 and 2007 and at Breda, Syria during 2007. Arrows shows the position of both parents of the population in the distribution.

trials. ILC 3182 flowered and matured early than ILC 3279. The reproductive period was longer for ILC 3182 at TH06 and TH07 as compared to ILC 3279. The duration of the trial at BR07 was longer than TH06 and TH07 because of much earlier sowing associated with slow early season crop growth due to cold weather. The earliest RIL flowered in 47 days at TH06, 54 days at TH07, and 92 days at BR07. The number of days to maturity ranged from 78 to 111 at TH06, 89 to 99 days at TH07, and 136 to 153 days at BR07. The reason for the narrow range in maturity during TH07 could have been the abrupt rise in temperatures late in the growing season. Plant height was greater at BR07 as compared to the other two trials. This may have been due to the longer vegetative period and favorable growing conditions during the vegetative period at BR07. Minimum plant height (17 cm) occurred at TH06, while maximum plant height (49 cm) occurred at BR07.

Harvest Index

The means, minimum and maximum along with standard error for parental genotypes and RILs for harvest index is provided in Table 2. The frequency distribution for harvest index in the three trials is presented in Fig. 5. The parents differed significantly for harvest index in all three trials. The range observed in TH06 trial was greater as compared to the other trials. In general, the harvest indices at BR07 trial were lowest with a range of 0.1 to 0.3, while the highest range was obtained during TH07 (0.3 – 0.6).

Table 2: Means, standard error and range of the RILs of the cross ILC 3182 x ILC 3279 as well as means for parental genotypes for various morphological traits under field drought stress conditions during 2006 and 2007.

Trait ^a	Trial ^b	# RILs	Parents		RILs			
			ILC 3182	ILC 3279	Min ^c	Max	Mean	Se (+/-)
HI	TH06	121	0.46	0.05	0.05	0.55	0.38	0.01
	TH07	106	0.48	0.35	0.28	0.61	0.44	0.01
	BR07	80	0.20	0.20	0.09	0.33	0.18	0.01
DTS	TH06	121	4.0	8.5	2.0	8.5	4.9	0.1
	TH07	106	4.5	7.1	2.9	7.1	4.8	0.1
	BR07	80	4.0	5.7	2.9	6.1	4.1	0.1
DFF	TH06	121	56	64	47	64	55	0.4
	TH07	106	58	69	54	69	60	0.3
	BR07	80	98	102	92	104	98	0.3
DM	TH06	121	92	94	78	111	95	0.8
	TH07	106	90	99	89	99	92	0.2
	BR07	80	146	153	136	153	146	0.4
RP	TH06	121	36	30	27	52	40	0.6
	TH07	106	25	24	24	37	32	0.3
	BR07	80	49	51	40	55	48	0.3
PH	TH06	121	36	32	17	37	28	0.3
	TH07	106	22	37	19	38	29	0.4
	BR07	80	34	48	31	49	42	0.5

^a DFF, number of days from sowing to first flower (when 50% plants of line showed first flower); DM, number of days from sowing to physiological maturity; RP, number of days between flowering and physiological maturity; PH, plant height (cm); HI, harvest index; DTS, Drought tolerance score on 1 to 9 scale where 1 is drought tolerant and 9 is drought susceptible

^b TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

^c Min, minimum, Max, maximum, se(+/-), standard error (+/-), SD, standard deviation

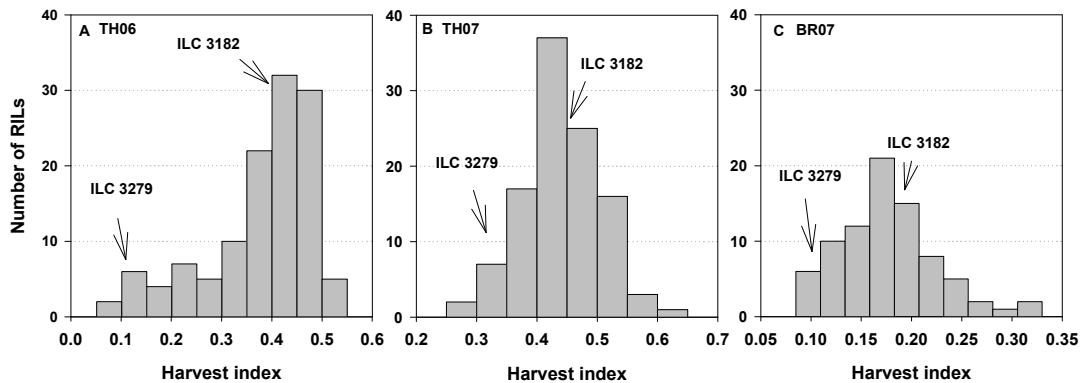


Fig. 5: Frequency distribution of RILs of a cross of ILC 3182 x ILC 3279 for harvest index for trials conducted at Tel Hadya, Syria during 2006 (A) and 2007 (B) and at Breda, Syria during 2007 (C). Arrows show the position of both parents of the population in the distribution.

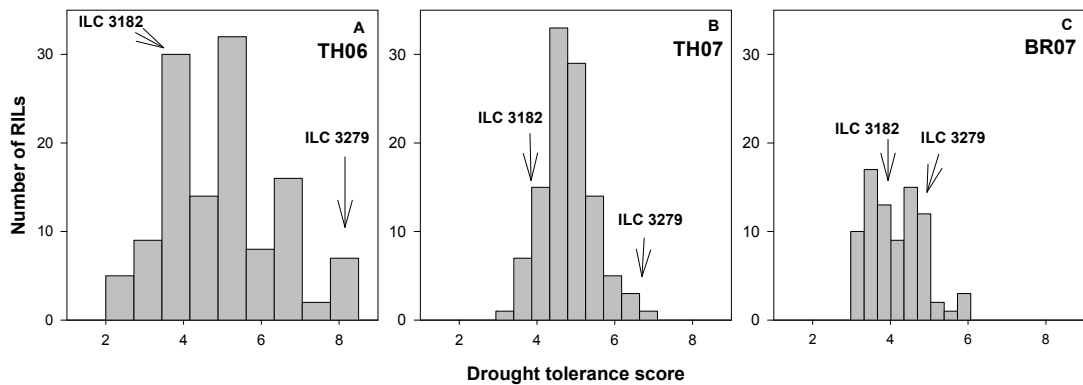


Fig. 6: Frequency distribution of RILs of a cross of ILC 3182 x ILC 3279 for drought tolerance score (DTS) for trials conducted at Tel Hadya, Syria during 2006 (A) and 2007 (B) and at Breda, Syria during 2007 (C), where 1 is drought tolerant and 9 is drought susceptible. Arrows show the position of both parents of the population in the distribution.

Drought tolerance score (DTS)

The means, minimum and maximum along with standard error for parental genotypes and RILs for drought tolerance score is provided in Table 2. The frequency distribution for drought tolerance score in all the three trials is presented in Fig. 6. The parents differed significantly for DTS in all the three trials. Drought tolerance score for ILC 3182 was nearly the same in all three trials (4.5 in TH07, 4.0 in TH06 and BR07), while DTS observed for ILC 3279 was much greater at BR07 (5.7) to 8.5 & 7.0 at TH06 & TH07, respectively. The better DTS observed for ILC 3279 during BR07 may have been due to the favourable environmental conditions during the vegetative period which resulted in substantial biomass accumulation.

Heritability estimates

The heritability estimates and standard error of estimates for grain yield and yield components are provided in Table 3, and for several important physiological and phenological traits in Table 4. The heritability was higher for grain yield (0.53 ± 0.03) and SDW (0.21 ± 0.06) at TH06 as compared to TH07 and BR07. Heritability estimates were higher for HI (0.70 ± 0.01) and DTS (0.70 ± 0.01) than for grain yield or SDW. Heritability estimates were higher at TH06 for HI and DTS as compared to the other trials. Similarly, heritability estimates were higher for DFF (0.61 ± 0.02) and DM (0.75 ± 0.01) at TH06 trial as compared to the other trials.

Relationship of traits with yield performance under drought

Phenotypic correlation coefficients among agro-morphological traits measured at the three trials are presented in Table 5 for TH06 trial, Table 6 for TH07 trial and Table 7 for BR07 trial. A graphical representation of the relationship between drought tolerance score (DTS) and grain yield is presented in Fig. 7, between shoot biomass and grain yield in Fig. 8 and between harvest index and grain yield in Fig. 9. Data showed a significant correlation between DTS and grain yield (g plant^{-1}) at TH06 (-0.66, $P < 0.01$), TH07 (-0.49, $P < 0.01$) and BR07 (-0.56, $P < 0.01$). DTS was also correlated significantly with HI, number of grains per plant and number of pods per plant at three trials. Shoot dry weight (SDW) was positively correlated with grain yield at TH06 (0.74, $P < 0.01$), TH07 (0.85, $P < 0.01$) and BR07 (0.64, $P < 0.01$). Harvest index was also correlated positively with grain yield at TH06 (0.79, $P < 0.01$), TH07 (0.62, $P < 0.01$) and BR07 (0.79, $P < 0.01$). Days to flowering was negatively correlated with grain yield in all three trials; correlation coefficients were significant in the trials with the exception of BR07. Similarly, days to maturity was also negatively correlated with grain yield; correlation coefficients were significant in all trials with the exception of TH07. Correlation coefficients were also significant for days to flowering and maturity with harvest index in all the three trials. This relationship was negative showing the importance of early flowering and maturity in escaping terminal drought stress.

Table 3: Broad-sense heritability estimations for grain yield and yield components measured in three trials during 2006-07 for chickpea recombinant inbred lines of the cross ILC 3182 x ILC 3279.

Trait ^a	Trial ^b	σ_g^c	σ_e	$h^2 \pm se$
Grain Yield	TH06	0.72	0.64	0.53 \pm 0.03
	TH07	0.53	2.46	0.18 \pm 0.05
	BR07	0.28	0.62	0.31 \pm 0.06
	Combined	0.11	1.43	0.17 \pm 0.15
SDW	TH06	0.89	3.27	0.21 \pm 0.06
	TH07	1.46	7.71	0.16 \pm 0.05
	BR07	1.60	8.63	0.16 \pm 0.06
	Combined	0.76	6.89	0.36 \pm 0.15
100-GW	TH06	5.45	6.67	0.45 \pm 0.04
	TH07	6.53	7.28	0.47 \pm 0.03
	BR07	3.90	16.80	0.19 \pm 0.05
	Combined	4.48	9.96	0.69 \pm 0.09
# of grains plant ⁻¹	TH06	13.21	11.96	0.52 \pm 0.03
	TH07	10.36	39.54	0.21 \pm 0.05
	BR07	5.48	10.20	0.35 \pm 0.05
	Combined	1.95	23.65	0.21 \pm 0.14
# of pods plant ⁻¹	TH06	13.61	11.43	0.54 \pm 0.06
	TH07	10.48	38.56	0.21 \pm 0.05
	BR07	6.44	15.88	0.29 \pm 0.06
	Combined	2.96	24.91	0.30 \pm 0.14

^a Yield, Grain yield (g plant⁻¹); SDW, shoot dry weight (g plant⁻¹); 100-GW, hundred grain weight (g); Grain P⁻¹, number of grains per plant; Pods P⁻¹, number of pods per plant; Grain Pod⁻¹

^b TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

^c σ_g , Genotypic variance; σ_e , environmental variance; $h^2 \pm se$, heritability estimates +/- standard error

Table 4: Broad-sense heritability estimations for important physiological and morphological traits measured in three trials during 2006-07 for chickpea recombinant inbred lines of the cross ILC 3182 x ILC 3279.

Trait	Trial ^b	σ_g^c	σ_e	$h^2 \pm se$
HI	TH06	0.01	0.00	0.70 ± 0.01
	TH07	0.00	0.01	0.20 ± 0.05
	BR07	0.00	0.00	0.41 ± 0.04
	Combined	0.00	0.00	0.54 ± 0.13
DTS	TH06	1.63	0.68	0.70 ± 0.01
	TH07	0.35	0.48	0.42 ± 0.03
	BR07	0.28	0.57	0.33 ± 0.05
	Combined	0.27	0.58	0.43 ± 0.13
DFF	TH06	11.82	7.46	0.61 ± 0.02
	TH07	6.35	4.83	0.57 ± 0.02
	BR07	4.99	3.76	0.57 ± 0.03
	Combined	5.43	5.37	0.68 ± 0.08
DM	TH06	62.96	21.30	0.75 ± 0.01
	TH07	1.83	1.71	0.52 ± 0.03
	BR07	10.02	9.03	0.53 ± 0.03
	Combined	11.87	9.70	0.53 ± 0.13
PH	TH06	8.55	9.63	0.47 ± 0.04
	TH07	11.37	8.26	0.58 ± 0.02
	BR07	14.52	4.54	0.76 ± 0.01
	Combined	10.54	7.56	0.87 ± 0.04

^a HI, harvest index; DTS, drought tolerance score on 1-9 scale; DFF, number of days from sowing to first flower (when 50% plants of line showed first flower); DM, number of days from sowing to physiological maturity; PH, plant height (cm)

^b TH06, trial location Tel Hadya, Syria during 2006; TH07, trial location Tel Hadya, Syria during 2007; BR07, trial location at Breda, Syria during 2007

^c σ_g , Genotypic variance; σ_e , environmental variance; $h^2 \pm se$, heritability estimates +/- standard error

Table 5: Phenotypic correlation coefficients among agro-morphological traits measured on a population of RILs from a cross between ILC 3182 x ILC 3279 under drought locations at Tel Hadya, Syria during 2006.

Traits ^a	DM	RP	DTS	SDW	GY	HI	100-GW	Grain P ⁻¹	Pods P ⁻¹	Grain Pod ⁻¹
DFF	0.78**	0.46**	0.79**	-0.1	-0.55**	-0.77**	-0.01	-0.58**	-0.60**	0.27**
DM		0.91**	0.87**	-0.17	-0.60**	-0.73**	0.00	-0.62**	-0.65**	0.27**
RP			0.72**	-0.17	-0.49**	-0.54**	0.02	-0.50**	-0.54**	0.21*
DTS				-0.21*	-0.66**	-0.82**	-0.08	-0.67**	-0.70**	0.29**
SDW					0.74**	0.22**	0.29**	0.69**	0.69**	-0.08
GY						0.79**	0.33**	0.93**	0.92**	-0.09
HI							0.23**	0.75**	0.74**	-0.13
100-GW								0.00	0.02	-0.18*
Grain P ⁻¹									0.98**	-0.05
Pods P ⁻¹										-0.19*

^a DFF, days from sowing to first flower; DM, days from sowing to maturity; RP, days between time of first flower and physiological maturity; DTS, drought tolerance score on 1-9 scale; SDW, shoot dry weight (g plant⁻¹); Yield, grain yield (g plant⁻¹); HI, harvest index; 100-GW, hundred grain weight (g); Grain P⁻¹, number of grains per plant; Pods P⁻¹, number of pods per plant; Grain Pod⁻¹, number of grains per pod

Table 6: Phenotypic correlation coefficients among agro-morphological traits measured on a population of RILs from a cross between ILC 3182 x ILC 3279 under drought locations at Tel Hadya, Syria during 2007.

Traits ^a	DM	RP	DTS	SDW	GY	HI	100-GW	Grain P ⁻¹	Pods P ⁻¹	Grain Pod ⁻¹
DFF	0.44**	-0.06	0.41**	-0.03	-0.25**	-0.41**	-0.09	-0.19*	-0.25**	0.13
DM		-0.07	0.34**	0.16	-0.09	-0.39**	-0.16	0.02	-0.01	0.09
RP			-0.13	0.24**	0.18	-0.02	-0.08	0.25**	0.24**	0.08
DTS				-0.32**	-0.49**	-0.44**	-0.17	-0.40**	-0.43**	0.03
SDW					0.85**	0.14	0.27**	0.79**	0.75**	0.24**
GY						0.62**	0.46**	0.83**	0.79**	0.31**
HI							0.44**	0.44**	0.40**	0.28**
100-GW								-0.09	-0.10	0.03
Grain P ⁻¹									0.95**	0.36**
Pods P ⁻¹										0.07

^a DFF, days from sowing to first flower; DM, days from sowing to maturity; RP, days between time of first flower and physiological maturity; DTS, drought tolerance score on 1-9 scale; SDW, shoot dry weight (g plant⁻¹); Yield, grain yield (g plant⁻¹); HI, harvest index; 100-GW, hundred grain weight (g); Grain P⁻¹, number of grains per plant; Pods P⁻¹, number of pods per plant; Grain Pod⁻¹, number of grains per pod

Table 7: Phenotypic correlation coefficients among agro-morphological traits measured on a population of RILs from a cross between ILC 3182 x ILC 3279 under drought locations at Breda, Syria during 2007.

Traits ^a	DM	RP	DTS	SDW	GY	HI	100-GW	Grain P ⁻¹	Pods P ⁻¹	Grain Pod ⁻¹
DFF	0.65**	-0.08	0.41**	0.36**	-0.07	-0.38**	-0.05	-0.06	0.01	-0.13
DM		0.71**	0.52**	0.17	-0.31**	-0.58**	-0.02	-0.33**	-0.19	-0.33**
RP			0.29**	-0.1	-0.33**	-0.39**	0.02	-0.37**	-0.24*	-0.31**
DTS				-0.23*	-0.56**	-0.55**	-0.18	-0.54**	-0.48**	-0.34**
SDW					0.64**	0.08	0.32**	0.53**	0.70**	0.07
GY						0.79**	0.45**	0.87**	0.86**	0.39**
HI							0.29**	0.74**	0.58**	0.54**
100-GW								-0.01	0.27*	-0.35**
Grain P ⁻¹									0.83**	0.64**
Pods P ⁻¹										0.14

^a DFF, days from sowing to first flower; DM, days from sowing to maturity; RP, days between time of first flower and physiological maturity; DTS, drought tolerance score on 1-9 scale; SDW, shoot dry weight (g plant⁻¹); Yield, grain yield (g plant⁻¹); HI, harvest index; 100-GW, hundred grain weight (g); Grain P⁻¹, number of grains per plant; Pods P⁻¹, number of pods per plant; Grain Pod⁻¹, number of grains per pod

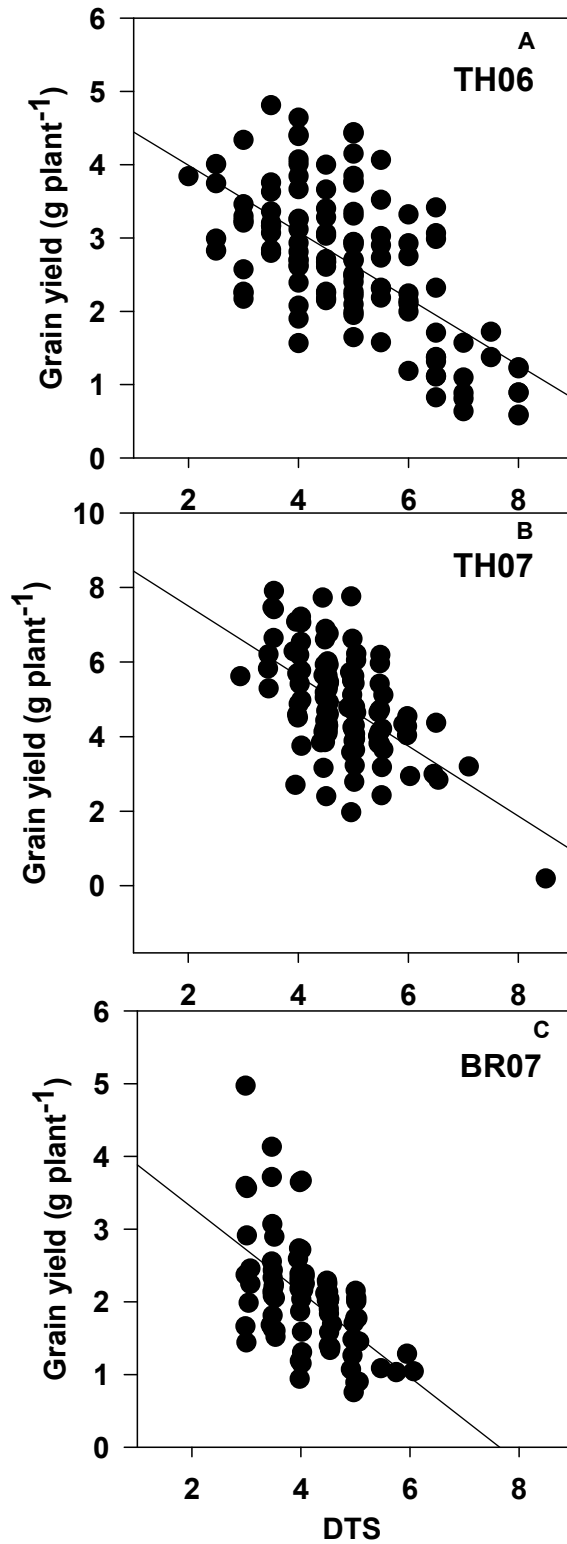


Fig. 7: The relationship of drought tolerance score (DTS) with grain yield (g plant⁻¹) under Mediterranean drought environments at (A) Tel Hadya, Syria during 2006 (TH06), (B) Tel Hadya, Syria during 2007 (TH07) and (C) Breda, Syria during 2007 (BR07).

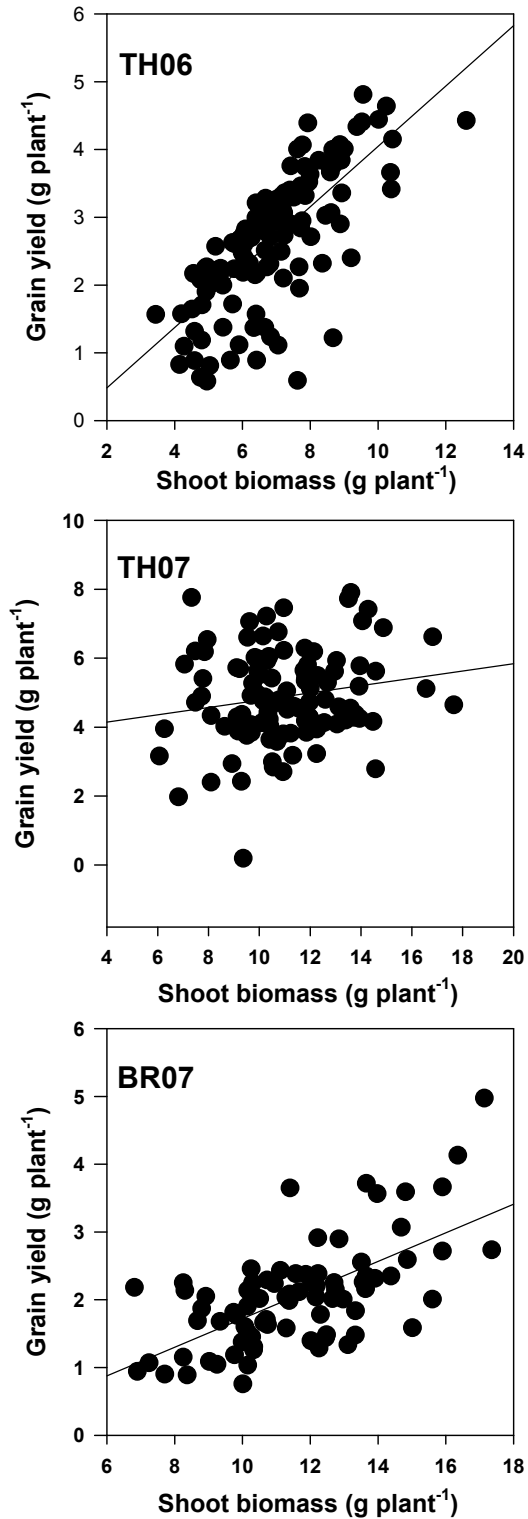


Fig. 8: The relationship of shoot biomass (g plant⁻¹) with grain yield (g plant⁻¹) under Mediterranean drought environments at (A) Tel Hadya, Syria during 2006 (TH06), (B) Tel Hadya, Syria during 2007 (TH07) and (C) Breda, Syria during 2007 (BR07).

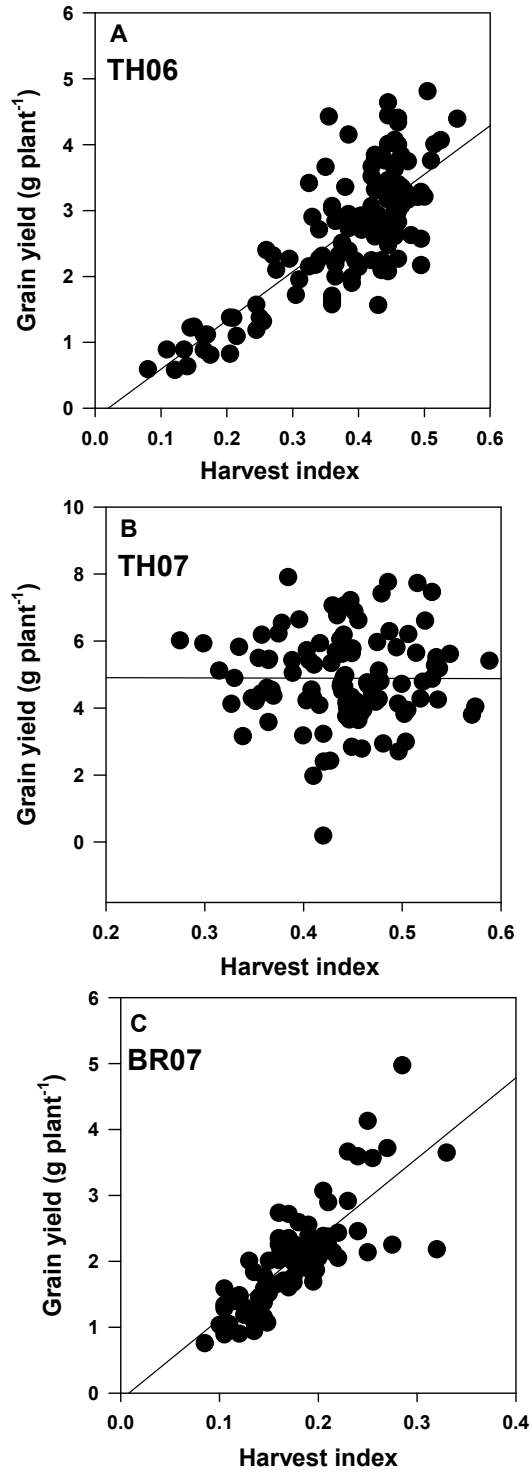


Fig. 9: The relationship of harvest index with grain yield (g plant⁻¹) under Mediterranean drought environments at (A) Tel Hadya, Syria during 2006 (TH06), (B) Tel Hadya, Syria during 2007 (TH07) and (C) Breda, Syria during 2007 (BR07).

Discussion

Most of the crop growth period at TH06 was drier than 2007. Only 26 mm precipitation was received during trial period at TH06 while 73 mm precipitation was received at TH07. Precipitation received at BR07 was 191 mm during trial period with majority of precipitation received during early plant growth (January / February). Mean grain yield (g plant^{-1}) of RILs of a cross of ILC 3182 x ILC 3279 at TH06 was 2.65 (g plant^{-1}), at TH07 it was 4.92 g plant^{-1} while lower grain yield was obtained during BR07 (2.06 g plant^{-1}). Similar trend was obtained at the three locations with RIL population of a cross of ILC 588 x ILC 3279 (chapter 5). Both populations produced higher shoot dry weight at Breda (BR07) than other two trials primarily because of longer vegetative period and good precipitation received during vegetative period at Breda site. This higher biomass obtained at this location could not translate into higher grain yield, probably due to drought stress and high temperature after flowering. This was also evident from HI data at three locations from both populations. Mean HI for both populations significantly reduced at BR07 (mean HI <0.19) as compared to TH06 (mean HI >0.38) and TH07 (mean HI >0.41). Range of HI for RIL population of ILC 3182 x ILC 3279 obtained during TH06 was 0.05 to 0.55, during TH07 was 0.28 to 0.61 while during BR07 it was 0.09 to 0.33. Similarly, range of HI for RIL population of ILC 588 x ILC 3279 obtained during TH06 was 0.02 to 0.59, during TH07 was 0.20 to 0.56 while during BR07 it was 0.06 to 0.33. Parental genotypes for these two RIL populations have lower HI than some RILs (ILC 3279 = HI range 0.05 – 0.35; ILC 588 = HI range 0.24 – 0.50; ILC 3182 = HI range 0.20 – 0.48) suggested a transgressive segregation for this trait. Heritability estimates for HI were also higher for both populations ($h^2 = 0.41 - 0.70$) with the

exception of TH07 where h^2 was in a range of 0.20 to 0.31 for both populations under three trials. Hay (1995) reviewed the role of harvest index in enhancing crop productivity in most cereal crops and suggested its use in pulse crops for yield improvement. Interestingly, in indeterminate crops like chickpea, the balance between vegetative and reproductive development can vary considerably according to the degree and timing of stress (Hay, 1995). The duration of the growth of chickpea crop grown in drier areas in India and Mediterranean region is cut short by drought (commonly less than 100 days). Severe drought coupled with high temperature can affect flowering process and reproductive period and ultimately affect HI. Hence, HI may prove to be useful index of the response of crops to climatic change. There is further need to understand the role of HI in chickpea improvement under less favourable environments. Increase in magnitude and stability of HI under marginal environments might help enhancing chickpea productivity.

Significant phenotypic correlations were detected for drought tolerance score (DTS) with grain yield ($r = 0.49 - 0.66$ $P < 0.01$) and harvest index ($r = 0.44 - 0.82$ $P < 0.01$) in all three trials suggesting the use of DTS for ranking genotypes under drought stress. DTS is a visual score which is easy to record prior to maturity. Similar relationship was also found when these traits were tested under drought with a RIL population of ILC 588 x ILC 3279 (chapter 5). Days to flowering and maturity were also significantly correlated (negative) with grain yield and HI suggesting the importance of early flowering and maturity in escaping terminal drought stress. Similar relationships were also detected among these traits in a RIL population of ILC 588 x ILC 3279 (chapter 5). Ludlow and Muchow (1990) also reported a strong interaction of plant phenology with

the expression of drought tolerance and yield potential. Early flowering and maturity are important in escaping a terminal drought stress. Genotypes with early flowering and maturity demonstrated trends of producing higher grain yield and HI under stress. Therefore, earliness is an advantageous trait under conditions of terminal drought stress. Higher number of pods and grains per plant as well as higher harvest index were also important contributors for higher grain yield under drought stress. Berger *et al.* (2006) confirmed the importance of high HI and drought escape through early flowering, podding and maturity of chickpea under natural drought stress. This finding agrees with the role of earliness as a drought escape mechanism in other crops including wheat and barley under Mediterranean environments (Acevedo *et al.*, 1991; González *et al.*, 1999).

This study revealed that improved grain yield under drought conditions in Mediterranean environments was associated with higher harvest index, early flowering and early maturity. The identification of quantitative trait loci (QTLs) for the key traits responsible for improved productivity under drought is recommended and could be helpful in accelerating the process of pyramiding of favorable genes into adapted genotypes for higher production under drought conditions.