ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

ECOPHYSIOLOGICAL INVESTIGATION ON EFFECT OF WATER STRESS ON EIGHT LOCAL CHICKPEA ACCESSIONS (*Cicer arietinum* L.)

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ABSTRACT

Chickpea (*Cicer arietinum* L.) plants were subjected to three watering regimes in order to evaluate effects of water stress on growth parameters, water relations, chlorophyll *a* fluorescence of PSII and drought induced gene expression under glasshouse conditions. A total of eight accessions were considered for the growth and physiological studies.

Water stress result in reductions of plant height branch number, leaf number, biomass accumulation, total leaf area and relative growth rates. Biomass allocation to leaves, stem and root did not differ between well-watered and stressed plants. Biomass partitioning to pods showed an increasing trend with stress level but it was not significant except in one accession (Ac3). Stomatal conductance declined with stress. Leaf water potential reached as low as -2.5 MPa in severely stressed plants, which had relative water content (RWC) between 50 - 60%. Analysis of chlorophyll *a* fluorescence showed that maximal photochemical yield (Fv/Fm) was not affected by water stress. However, there was a slight decrease in Fv/Fm ratio in the dark. On the other hand, quantum efficiency (ΔF/Fm') declined substantially under water stress in most accessions. Moreover, diurnal reduction in Fm (maximum fluorescence) was observed indicating the existence of mechanisms for thermal dissipation of excess energy at high irradiance. Water stress reduced seed yield by reducing the number of pods per plant. However, number of seeds per pod was not affected although smaller seeds were harvested in severely stressed plants. It is concluded that out of the studied physiological attributes, stomatal conductances, RWC, quantum efficiency and cell membrane stability tests showed intraspecific variations indicating that they could be used for selection of resistant types.

1. INTRODUCTION

1.1. Scope of the problem

Eventhough water is the most abundant compound on earth, its shortage is the most important abiotic factor that limits crop productivity and distribution of species in the world. In the past decade, the occurrence of drought in the world in general and in Sub-sharan Africa in particular has severely reduced agricultural production. In Ethiopia, for example, drought has occurred more than once during the past ten years. In the year 1987 alone, drought had occurred in the northern part of Ethiopia including most parts of Shoa, Hararghe and the Rift Valley, where it is reported to have affected 5.2 million people (RRC, 1987). One possible solution to increase productivity of crop plants is to use irrigation. However, the water for irrigation purpose may not be always available. Moreover, artificial irrigation may lead also to permanent damage in the form of soil salinization (McWilliam, 1986). One of the most practical and economical approaches is to select and/or develop drought tolerant cultivars that give high yield under limited water supply. Among the food legumes cultivated in Ethiopia, Chickpea is the most important pulse crop that suffers from terminal drought (Engels et al., 1989). Thus, breeding for drought tolerance is a major objective in Chickpea improvement program.

Chickpea (*Cicer arietinum* L.) is one of the most important food legumes in the semi-arid tropics. Although the origin of chickpea is believed to be south-western Asia, it grows in many parts of the world including Ethiopia (Dombrowski, 1969; Westphal, 1974; Purseglove, 1976). In Ethiopia, chickpea is cultivated to a large extent at altitudes between 1400-2800 m where annual rainfall ranges between 700 and 2000 mm (Taye Bizuneh, 1975; Seme Debela et al., 1987; Tamirie Hawando, 1987; Hailu Mekbib et al., 1991). Ethiopia possess more than one third of the total area cultivated with chickpea in Africa (135000 ha), and nearly half of the total production (265000 tons) (FAO, 1992).

Ethiopia is one of the major centres of diversity for chickpea (Engels et al., 1989; Hailu Mekbib et al., 1991; Seme Debela et al., 1987). At present there are about 900 accessions conserved at Institute of Biodiversity Conservation Research (IBCR) which were collected from diverse ecological zones in the central and northern parts of the country (Hailu Mekbib et al., 1991). Chickpea grows widely in these regions on residual moisture after the main rain season following the harvest of cereal crops (Taye Bizuneh, 1975; Hailu Mekbib et al., 1991). The average yield of this crop in Ethiopia ranges between 110-700 kg ha^{-1} which is lower than that of Sudan and Egypt which varies between 1000-1100 kg ha^{-1} and 1000-2000 kg ha^{-1}, respectively (Tamirie Hawando, 1987).

Many Ethiopians consume chickpea, as a protein supplement both in rural and the urban sector. In general, it is estimated to contain 17.4% protein, 5.4% fat and 63.9% carbohydrate (Agren and Gibson, 1968). Thus, when mixed with cereals chickpea can satisfy the human nutrition requirements. Dehulled (*kik*), boiled (*nifro*) and roasted (*kollo*) forms are some of the food preparations used traditionally in Ethiopia.

The productivity of chickpea has been fairly static for the last twenty years due to the susceptibility of contemporary varieties to a combination of biotic (plant factors, disease, insect pest and weeds) and abiotic (soil alkalinity, salinity, water logging, water deficit etc) factors (Seme Debela et al., 1987; Tamirie Hawando, 1987; Singh et al., 1994). Water stress is the major constraint to productivity of chickpea particularly in the late-vegetative and reproductive stages. It is believed to cause nearly 50% of the reduction in yield (Seme Debela et al., 1987; Silim et al., 1993).

On the other hand, drought tolerance is a complex trait involving a range of individual physiological features and processes. Different local varieties may be drought resistant for different reasons. This is the probable explanation for the very limited progress that has been made with breeding for drought tolerance using simple screening methods. Factors which will enhance crop performance under water deficit include: (1) increased exploitation of soil water, (2) increased efficiency in the use of water and (3) increased ability to maintain metabolism and assimilate allocation under water deficit. It is unlikely to find all these characteristics combined in a single genotype and hence the best prospect for producing drought tolerant cultivars will come from accumulating information for the various physiological features.

Several methods have been used to select resistant cultivars to moisture stress in other crop plants. One of the methods used by physiologist, as a tool for selecting drought tolerance is chlorophyll fluorescence. According to Demming and Björkman (1987), a reduction in optimal photosystem II (PSII) photochemical efficiency (Fv/Fm) of dark-adapted leaf may reveal either a damage in PSII or onset of protective mechanisms against excess light energy. Flagella et al. (1994) for instance, used inhibition of photochemical quenching (qP) during the fluorescence induction curve as a screening tool for drought resistance in wheat. Similarly, using the same crop plant, the degree of reduction of quantum efficiency has been found to correlate with drought susceptibility index (DSI) of cultivars evaluated on yield basis (Flagella et al. 1996). In general, this technique has an advantage since it is fast and nondestructive. Using this method it is possible to detect damage before visible symptoms are observed (Wilson and Greaves, 1990).

During water deficit, stomatal conductance has an important role in controlling the balance between CO_2 uptake and water vapour loss (El Hafid et al., 1998). For instance, Castonguay and Markhart (1992) reported that bean plants conserve and optimise the use of water under drought condition. As the result stomatal conductance or resistance has been used in selecting drought tolerant varieties. Stomatal conductance has been employed along with other parameters to screen drought tolerance in barley (Martin et al., 1989). In addition, use of relative water content and leaf water potential for screening purpose has long been recognised.

Damage to membrane by stress affects physiological processes including photosynthesis (Sullivan and Rose, 1979). Furthermore, the degree of injury is determined by ion leakage. Thus, membrane stability test has been used as an indicator of drought tolerance. Recently, Belay Shiferaw and Baker (1996) have employed this technique for selecting drought tolerant *tef* cultivars.

Physiological attributes have already been used in crop improvement programs in chickpea and other related crops (Farquhar et al., 1984; Frank et al., 1985; ICRISAT, 1988; 1993). Root dimension, flowering time, maturity period, shoot growth, biomass production and leaf area index were among the parameters used for screening chickpea cultivars (ICRISAT, 1988; 1993). Such investigations have shown that chickpea is not an efficient plant in terms of yield (Thomas and Fukai, 1995). This has been attributed mainly to inefficient use and partitioning of resources even under well-watered condition (Hooda et al., 1986).

An understanding of plant adaptation to drought requires, thus, an investigation of a wide range of physiological, biochemical, morphological and agronomic characters (Hooda et al., 1986; 1990; Singh et al., 1994; Jirali et al., 1994; Thomas and Fukai, 1995). Therefore, combined agronomical, physiological and molecular studies are needed to identify the traits that can contribute to chickpea productivity under water stress conditions. So far, most of the studies that have been conducted on chickpea in many countries, including Ethiopia, have been directed towards the improvement of cultivars that are high yielding under rainfed and irrigable conditions. Such cultivars perform poorly under water stress conditions (Seme et al., 1987). On the other hand, for the purposes of improving the performance of chickpea under drought, there exists little exploited genetic potential in the large number of locally adapted cultivars collected by the Institute of Biodiversity Conservation and Research (IBCR). The present project stems from this understanding. The rationale of the project objective is that a programme of selection and recombination is required to exploit this genetic potential for drought resistance.

1.2. Objective of the study

General objective:

To characterise and select agronomic and physiological traits in eight chickpea accessions, which can be used to select drought resistant varieties.

Specific objectives:

To investigate growth characteristics and physiological features of local chickpea under different water stress regimes.

To identify physiological, as well as agronomical parameters that can be used for selecting susceptible and tolerant varieties from among eight chickpea accessions under moisture stress.

2. MATERIALS AND METHODS

2.1. Plant material and growth conditions

The experiments were conducted in the greenhouse at Science Faculty, Addis Ababa University between April and September 1998. During the study period the environmental conditions in the greenhouse were not controlled, however, the daily temperature and relative humidity were recorded with hygrothermograph. The daily average maximum and minimum temperatures were 37.05 ± 3.0 and 14.81 ± 1.0 respectively. The relative humidity ranged between 87 ± 9.0 and 23 ± 4.0%. The average daily photosynthetic active radiation at 12:00 during sunny days in the greenhouse was 1750 ± 49 μ mol m^{-2} s^{-1} as measured with light meter.

Chickpea seeds (*Cicer arietinum* L., accessions no., 207148, 212589, 207894, 41212, 41095, 41102, 41007 and 207150, hereafter refered to as Ac1, Ac2, Ac3, Ac4, Ac5, Ac6, Ac7 and Ac8, respectively) were obtained from IBCR. The selection was based on the information available at the Institute. In addition, particular emphasis was given to accessions collected from drought prone areas (lowland). Some accessions from highland areas were also included. Seeds were sown in moist filter paper in Petridish and kept at room temperature for three days for germination. Seedlings were transferred to polyethylene bag (30 x 20 cm), containing 2.5 kg mixture of sand, compost (horse dung) and soil (clay) 4:2:1 (w/v) at a density of one seedling per pot. The pots were fertilized with 100 ml of complete Hoagland solution (Hewitt and Smith, 1975) every seven days for the first two weeks.

2.2. Treatment and experimental design

The seedlings were irrigated adequately every other day for the first three weeks until day 22. The weight of the pots when the soil was wetted to pot capacity was determined by applying 1050 ± 50 ml of water to the soil in the pots and allowing drainage for overnight. The average weight of the pots plus the soil at pot capacity was found to be 3533 ± 46 g. Seedlings were then subjected to three different water stress treatments following Hooda et al. (1990) with little modification.

a) Well-watered (WW) - Soil water content maintained at 60 - 70% pot capacity.
b) Moderately stressed (MS) - Soil water content maintained at 30 - 40% pot capacity.
c) Severely stressed (SS) - Soil water content maintained at 10 - 20% pot capacity.

Moisture loss was monitored daily by weighing five pots randomly from each treatment. Water lost from the pot was replenished daily to keep the soil at the three set levels of moisture.

The experiments were conducted following randomized complete block design with three replications. Each block consisted 18 pots (6 per treatment) for the determination of various parameters.

2.3. Data collection

2.3.1. Growth parameters

Before the beginning of the stress treatment (18 DAP) plant height, leaf number and branch number were measured for all plants.

2.3.2. Plant height

Plant height of three randomly selected plants per treatment was measured from the base to highest point of the plant on 37 DAP and 57 DAP. The average mean height was then recorded.

2.3.3. Leaf and branch number

Leaf and total branch number (primary, secondary and tertiary) from three randomly selected plants per treatment were recorded 37 DAP and 57 DAP.

2.3.4. Biomass, biomass partitioning and root/shoot ratio

Plants were sampled during the experiments to determine biomass in various plant components. The first and second harvests were made from three randomly selected plants on 24 DAP and 42 DAP, respectively. Each plant was uprooted from the pots and separated into leaf, stem including branch, pod and root. Roots were collected in a 1 mm sieve after washing them free of soil with tap water.

Before drying, the leaf area of five randomly selected leaf was determined by an area meter (Delta T devices Ltd., Cambridge, England). Later the method of Adjei-Twum and Splittstorsser (1976) with little modification (see Masresha Fetene, 1985) was used to calculate the total leaf area. The ratio of the mean sample leaf area (A) to dry weight of the sample leaf (B) was used to obtain total leaf area of the individual plant:

Total leaf area =(A/B)*total dry leaf weight

The oven dry weight of all plant parts was recorded after drying at 80°C to constant weight. Hereafter, biomass partitioning will refer to the distribution of biomass between organs of the plant, expressed as a percentage of biomass of the whole plant. Root/shoot ratio was also calculated.

2.3.5. Relative growth rate

The mean relative growth rate (RGR) were estimated following Radford (1967) as:

$$RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$$

Where W_1 and W_2 are estimated organ dry weights at the first (t_1) and second (t_2) measurements of the time interval $t_1 - t_2$.

2.3.6. Yield and yield components

When the plants reached maturity, the pods were removed (September 28, 1998) and counted from all plants. The pods were then oven-dried at 80°C to constant weight. Number of seeds per pod and seed weight per plant were recorded after threshing the pods.

2.3.7. Leaf water potential

Leaf water potential was measured using a pressure chamber (SKYE, SKPM1400, SKYE Instruments, Powys, Wales) following Scholander et al. (1965) and Masresha Fetene (1985). To minimize errors due to water loss during the procedure, branches from three randomly selected plants were cut with a razor blade and transported to the chamber wrapped in polyethylene bag. The cut end of the branch was immediately inserted from the underside of the lid of the chamber, with the cut end of the branch projecting outside. The chamber was pressured from compressed nitrogen source and the cut end of the branch was examined using hand lens. The pressure at the point at which sap began to exude from the stem was recorded. Because Laurie and Stewart (1993) reported that there was little variation in diurnal leaf water potential between 14:30 - 16:00 h in chickpea, the measurements were made at this time of the day.

2.3.8. Relative water content

Relative water content of leaf (RWC) was estimated as:

$$RWC = (FW - DW)/(SW - DW)*100$$

Where FW is the fresh weight of leaflets, SW is the saturated weight of the leaflets floated on distilled water in Petridish for 24 hours, and DW the dry weight of the leaflets oven dried at 80°C to constant weight. For this measurement, leaf from three randomly selected plants were excised and only the leaflets were used for the estimation of RWC.

2.3.9. Stomatal conductance

Measurement of stomatal conductance of the abaxial surfaces was made on 25 June, 6 July and 14 July 1998 on the 3rd or 4th upper most fully expanded leaf using a portable Dynamic Diffusive Porometer (AP4, Delta-T, Devices, Cambridge, England). Stomatal conductance readings from two randomly selected plants per block were recorded.

2.3.10. Chlorophyll *a* fluorescence

Determination of chlorophyll *a* fluorescence from PSII was made on attached 3rd or 4th upper most fully expanded leaf using portable fluorometer (Plant Efficiency Analyzer (PEA), Hansatech Instruments Ltd, King's Lynn, Norfolk, UK). Before each measurement, leaf samples were dark adapted for 30 min with non-translucent plastic leaf clips. The sensor was placed over the leaf clip and illuminated with high-intensity light emitting diodes (LED), emitting light at a wavelength of λ=650 nm. The sensor unit's central photodiode absorbs the fluorescence and automatically calculates the fluorescence indices Fo, Fm, Fv and the ratio Fv/Fm. Fo is the initial fluorescence (all PSII reaction centers are oxidized or open) and Fm the maximum fluorescence at P level, which is achieved at saturating light intensity (complete reduction of QA), of PSII reaction centers (Bolhär-Nordenkampf and Öquist, 1993).

A pulse-amplitude-modulated fluorometer (Mini-Pam, H. Walz, Effltrich, Germany) was used to monitor chlorophyll fluorescence for light adapted samples as well as to measure the status of electron transport rate (see Masresha Fetene et al., 1997). The fluorometer was connected to a leaf clip holder (2030B, Henz, Walz) and fiberoptic that form a 60° angle with the leaf plane. The leaf clip is equipped with sensors for simultaneous recording of photosynthetically active radiation (PAR) and a thermocouple monitoring leaf temperature (NiCr-Ni). The quantum efficiency of PSII photochemistry was estimated as Φe = (Fm' - F) Fm' = ΔF/Fm' (Genty et al., 1989), where Fm' is the maximal fluorescence yield measured at the end of saturating light pulse, and F is the fluorescence yield measured before the last saturating pulse triggered in light adapted leaf samples. The non-photochemical quenching (NPQ) (which reflects heat-dissipation of excitation energy in the antennae) was estimated as

Fm - Fm'/Fm' (Bilger and Björkmann, 1991). Similarly, electron transport rate (ETR) was calculated as:

ETR $=\Phi_{PSII}$*PAR*0.5*0.84 (Schreiber et al., 1994).

The actinic light intensity for light saturation curve was increased in 8 steps within 4 min at 30 s intervals. At each step a saturating pulse was triggered to estimate ΔF/Fm' and ETR.

2.3.11. Cell membrane stability test

The method of Blum and Ebercon (1982) was followed to test cell membrane stability. Leaflets (0.2 g) from well-watered plants were placed into 50 ml plastic tubes containing 10 ml of either double distilled water or 47% polyethyleneglycol (PEG) solution. The tubes were incubated at 14°C for 48 hours. After discarding the media, the samples were washed with double distilled water three times, then 10 ml of double distilled water was added and incubated at 14°C for 48 hours. The tubes were equilibrated to 25°C with waterbath and the conductivity of the solution was measured with a conductance meter (Jenway, model 4071, UK). The tubes with the leaf samples were autoclaved at 120°C for 15 min to release, in this case, all the remaining electrolytes from the cell. After cooling tubes to 25°C, the contents were mixed and final conductance was measured. Based on the two conductance measurements, the relative injury was calculated as:

$$\text{Relative Injury [RI]\%} = [1 - (1 - (T_1/T_2))/(1 - (C_1/C_2))]*100$$

Where T and C refer to the conductance in treated and control tubes, and the subscripts 1 and 2 represent the readings before and after autoclaving, respectively.

2.3.12. Statistical analysis

All the data collected were analyzed with analysis of variance (ANOVA) test or t-test (SPSS/PC+, Statistical Package, version 7.5). The treatment means were compared by Tukey's Family Error Rate method at $P \leq 0.05$.

3. RESULTS

Similar trends in all parameters investigated were observed in all blocks, thus, the data were pooled together for analysis.

3.1. Vegetative growth

In general, water stress significantly affected vegetative growth of the chickpea accessions studied, including plant height, leaf number, branch number and total leaf area. The effect of water stress on plant height is shown in Fig. 1. Plant height was significantly reduced by water stress in all accessions. Plant height ranged from 33.9 to 37.4, 27.7 to 30.4 and 24.7 to 28.0 cm in optimally watered, moderately and severely stressed plants, respectively, 57 DAP. However, no significant differences among accessions were found both under control and stressed conditions.

Leaf and branch number showed similar pattern to plant height in both treatments. Leaf number per plant was lowered by 48 - 61% in moderately stressed plants and by 71 - 77% in severely stressed plants at last sampling date (57 DAP). Differences in leaf number between irrigated and stressed plants was not high at 37 DAP (Fig. 2). However, as the duration of the stress continued (57 DAP) the difference was more pronounced. No differences in leaf number were observed among accessions either when irrigated or when water stressed. Similarly, branch number was consistently higher in optimally watered plants than in stressed plants on both sampling occasions (Fig. 3).

Total leaf area per plant in the stressed plants was significantly lower in comparison to control plants (Fig. 4). Total leaf area was highest for accession Ac4 and lowest for accession

Ac5 under severe stress. However, variation among accessions was not detected in all treatments.

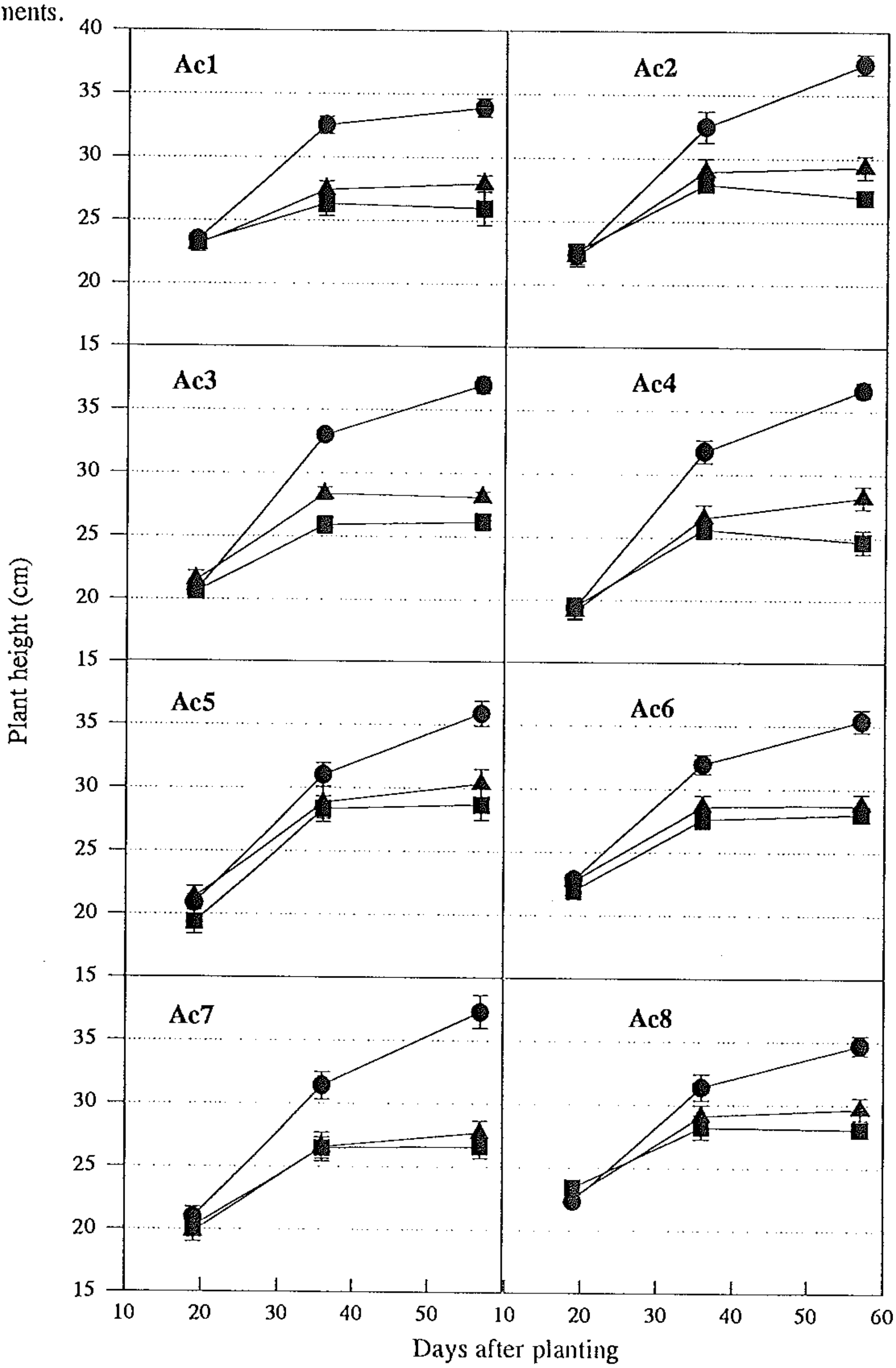


Fig. 1. Change in plant height of chickpea accessions under three moisture regimes: control (–●–), moderate stress (–▲–) and severe stress (–■–). Bars indicate S.E. of means (n=9-18).

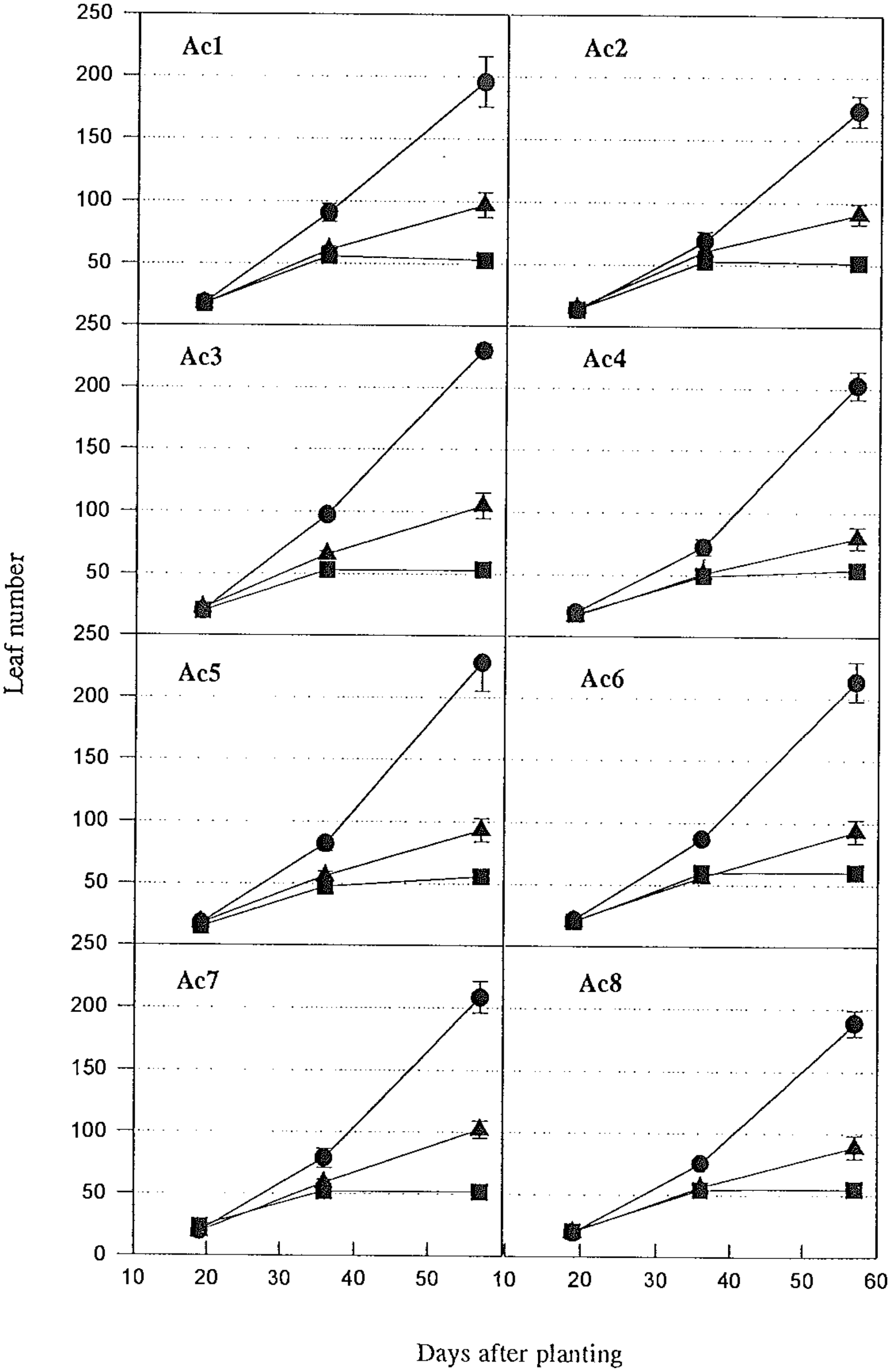
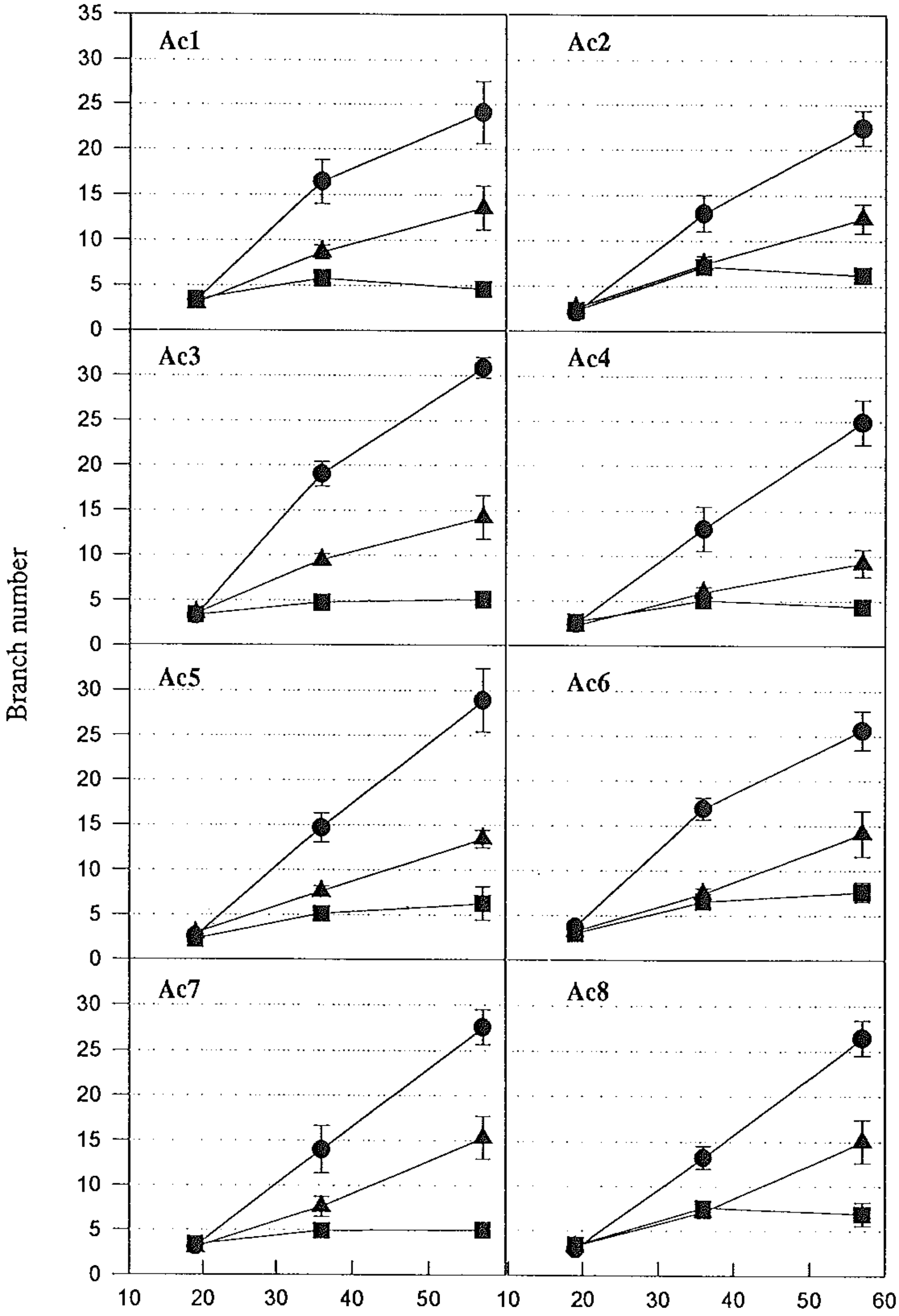


Fig. 2. Change in leaf number of chickpea accessions under three moisture regimes: control (–●–), moderate stress (–▲–) and severe stress (–■–). Bars indicate S.E. of means (n=9-18).



Days after planting

Fig. 3. Change in branch number of chickpea accessions under three moisture regimes:control (—●—), moderate stress (—▲—) and severe stress (—■—). Bars indicate S.E. of means (n=9-18).

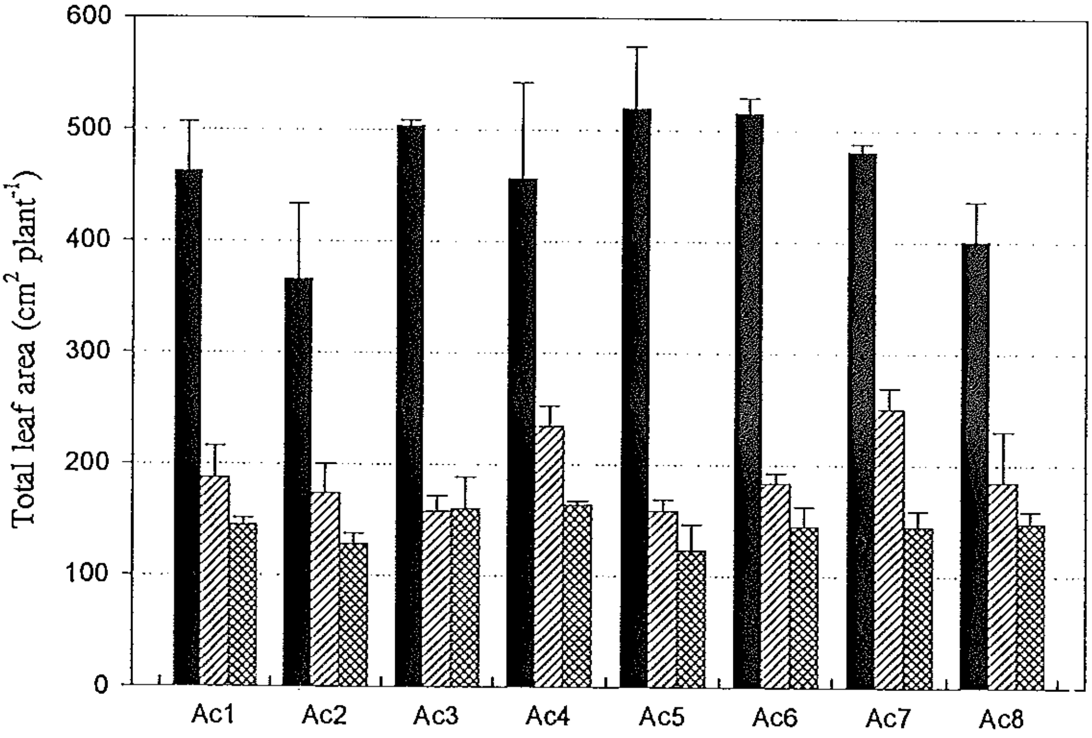


Fig.4. Total leaf area per plant of eight chickpea accessions measured 57 DAP under three moisture regimes: control (■), moderate stress (▨) and severe stress (▩). Bars indicate S.E. of means (n=3).

Biomass at 57 DAP for above ground parts is shown in Fig. 5a. Drought stressed plants had lower shoot biomass than control plants in all accessions except accession Ac2. Under moderately and severe stress conditions shoot biomass was reduced by 33 - 55% and 46 - 63%, respectively. No significant differences in shoot biomass were observed among accessions under both irrigated and stressed treatments. Root biomass production was affected by water stress as in shoots (Fig. 5b). However, the effect was more pronounced in roots than in shoots. Although it was statistically significant, accession Ac5 had the lowest root weight while the highest was recorded for by accession Ac4, under well-watered condition. Accessions Ac5 and Ac7 were the least affected under severe stress, which showed only 54 and 28% reduction in root biomass, respectively, perhaps due to their low biomass even under irrigated condition.

Plants grown in well watered soil had similar relative growth rates (RGR) as plants grown in drying soil in all accessions except Ac1, Ac2 and Ac5 (Fig. 6a). Furthermore, from 23 to 40 DAP, RGR was positive for above ground parts both under irrigated and stressed conditions. RGR in shoots was found to be highest for accession Ac4 in both well-watered and moderately stressed plants. The lowest RGR was shown by accession Ac5, contributing to their lower plant biomass (Fig. 5a;b). However, no significant differences in RGR in shoot were observed among accessions under all treatments. Similar trend was seen in RGR of roots with accession Ac4 showing highest RGR and Ac5 having smallest value under both optimally watered and severely stressed treatments (Fig. 6b).

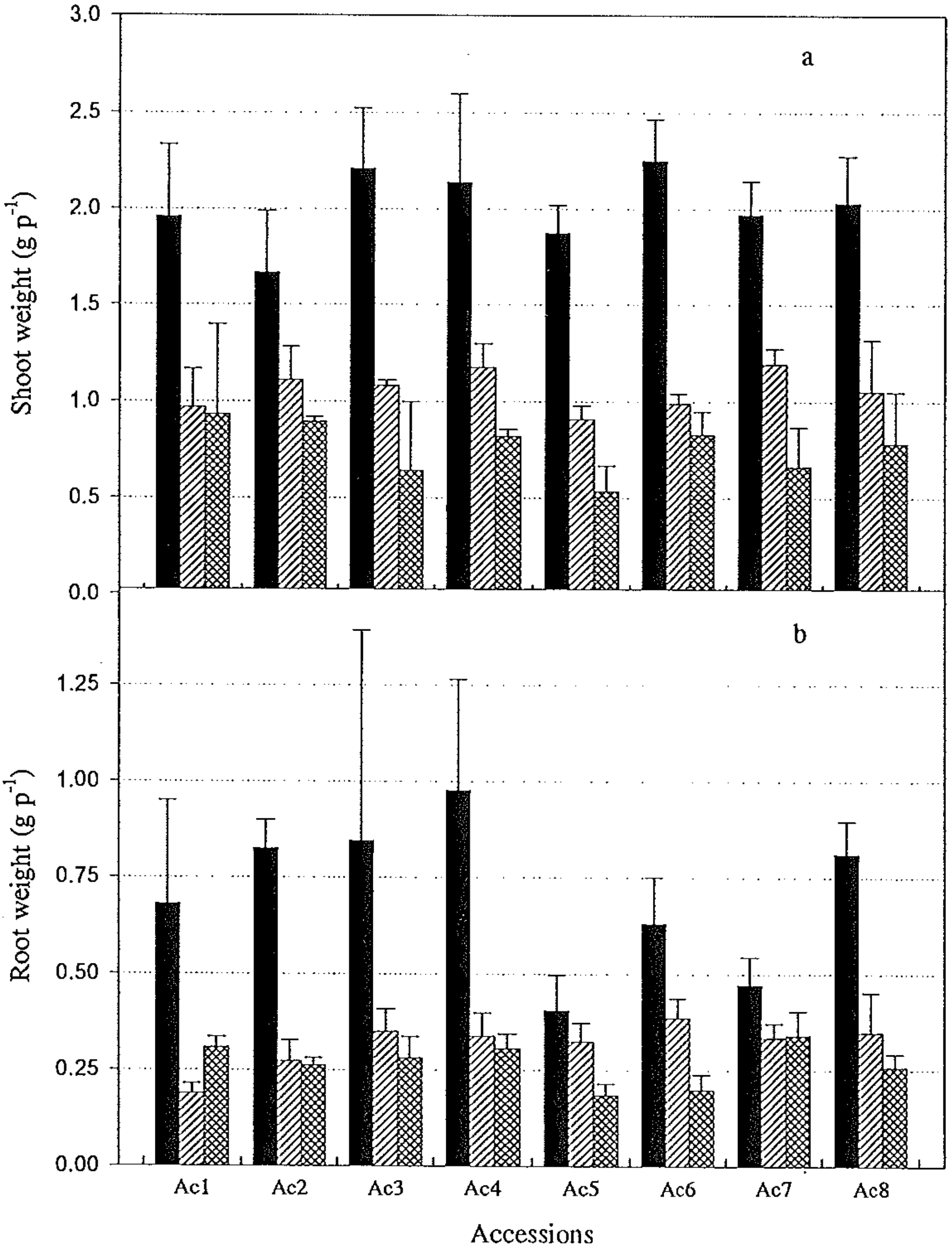


Fig. 5. Biomass of chickpea accessions in a) shoot and b) root under three moisture regimes: control (■), moderate stress (▨) and severe stress (▩). Bars indicate S.E. of means (n=3).

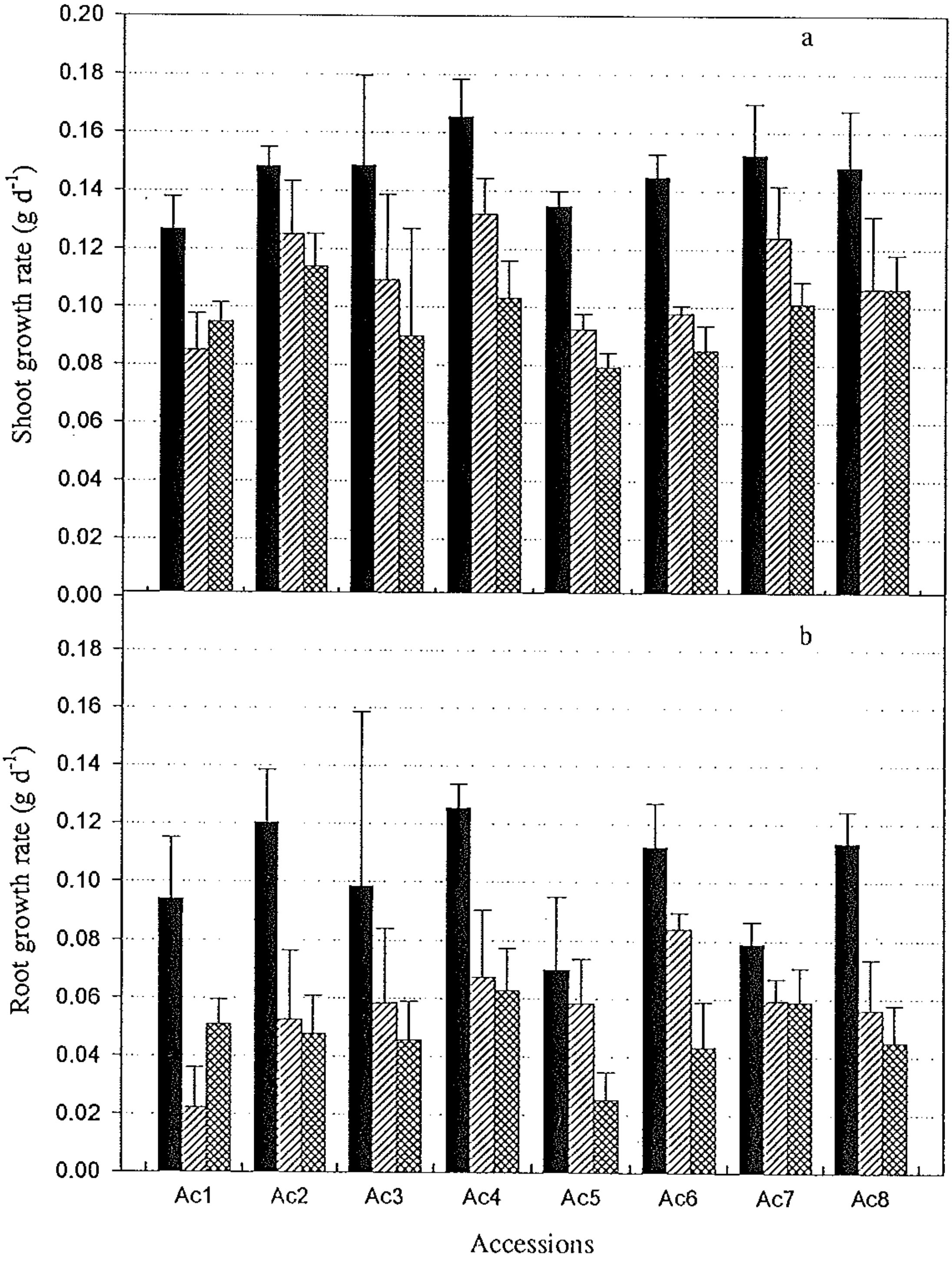


Fig. 6. Relative growth rate of a) shoot and b) root in eight chickpea accessions under three moisture regimes: control (■), moderate stress (▨) and severe stress (▩). Bars indicate S.E. of means (n=3).

Root/shoot ratio were similar for all watering levels except in accession Ac5 that showed a marked increase under moderately stressed condition (Fig. 7), which declined under severe stress level. In Ac7 root/shoot ratio increased from well watered to severely stressed plants. However the difference was not significant. Root/shoot ratio was similar among all accessions regardless of watering regimes.

Water stress (57 DAP) had no significant effect on partitioning of total biomass to leaf in all accessions except in Ac5. In accession Ac5 lower biomass was allocated to leaf in plants under stress than in the controls (Fig. 8a). Under moderate stress levels accession Ac1 showed higher (51.03%) total biomass allocation to leaf which was significantly different from accession Ac3 but not with other accessions. Percentage allocation to stems is shown in Fig. 8b. Biomass partitioning to stem was not affected significantly in stressed plants compared to controls. It accounted 22 - 29% of the total biomass. Similarly, partitioning of total biomass to roots did not differ in control and stressed plants in all accessions except in Ac6 that showed a 28% increase under moderate stress level, which later declined in severely stressed treatment (Fig. 8c). When percentage allocation to pods is considered, there was a general trend that showed an increase with stress treatment in most accessions. However, the increase was significant only in accession Ac3 (Fig. 8d). Accession Ac3 had the greatest percent allocation to its pods (17.69%) under moderately watered plants.

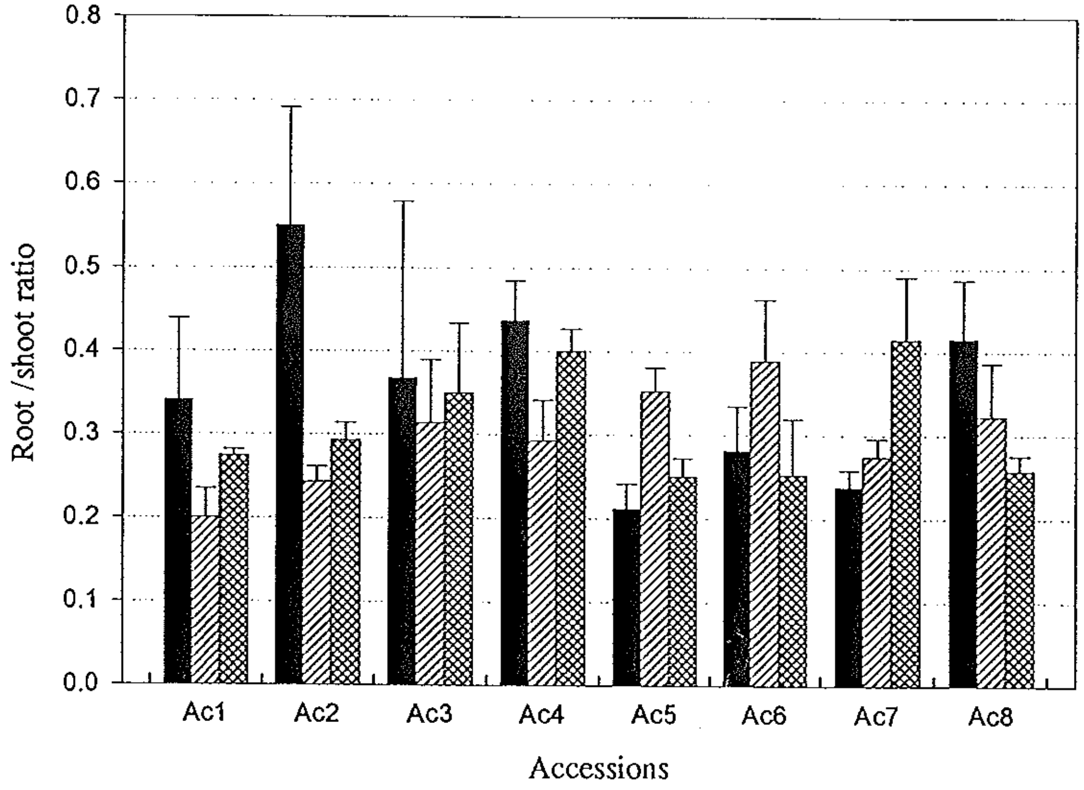


Fig. 7. Root/shoot ratio of eight chickpea accessions under three moisture regimes: control (■), moderate stress (▨) and severe stress (▩). Bars indicate S.E. of means (n=3).

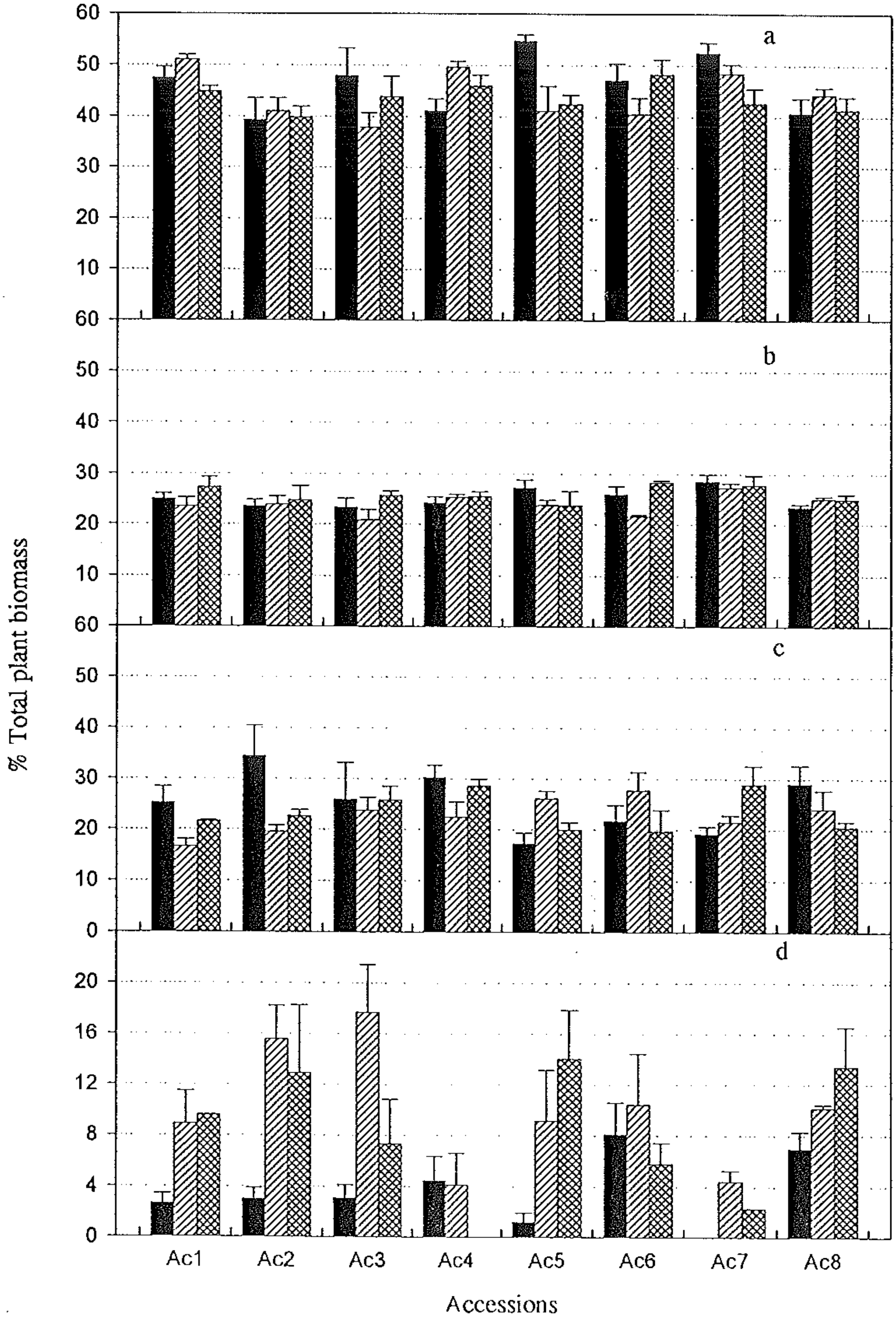


Fig. 8. Biomass partitioning into a) leaf , b) stem , c) root and d) pods expressed as percentage of total biomass in chickpea accessions under three moisture regimes (57 DAP): control (■), moderate stress (▨) and severe stress (▩). Bars indicate S.E. of means (n=3). Note change in scale in d.

3.2. Water relations

Relative water content (RWC) determinations are shown in Fig. 9. RWC under droughted condition was significantly lower than under well watered condition. There was no clear differentiation among accessions under optimally watered and moderately stressed plants. However, marked differences among accessions were observed under severe stress level with accession Ac1 showing the least value.

Result of leaf water potential measured between 14:30 - 16:00 h is shown in Table 1. Leaf water potential was smaller under water stress than under irrigated condition, and values ranged from -0.31 to -0.567 MPa (irrigated), -0.98 to -1.61 MPa (moderately stressed) and -2.26 to -2.57 MPa (severely stressed). Leaf water potential was not estimated for all accessions.

Table 1. Leaf water potential (-MPa) of eight chickpea accessions grown under three watering regimes. (n=2-4, mean±sd).

Accessions	Control	Moderate stress	Severe stress
Ac1	0.520 ± 1.40	1.548 ± 1.90	nd
Ac2	0.515 ± 1.30	1.606 ± 0.86	nd
Ac3	0.525 ± 0.63	1.410 ± 2.50	2.450 ± 0.90
Ac4	0.530 ± 0.39	0.978 ± 0.13	2.255 ± 1.50
Ac5	0.515 ± 1.64	1.262 ± 0.22	2.570 ± 0.30
Ac6	0.506 ± 0.21	1.219 ± 1.00	nd
Ac7	0.568 ± 0.04	nd*	2.350 ± 2.50
Ac8	0.311 ± 0.48	1.099 ± 0.68	nd

*= not determined

Exposure of chickpea plants to water stress significantly reduced the rate of loss of water vapour and increased stomatal resistance in all accessions except in accession Ac1 (Fig. 10). For comparison purpose only the morning conductances data (6:45 - 9:10 am) are reported. Under severe water stress condition, all accessions maintained smaller stomatal conductance and the minimum conductance was observed in accessions Ac3 and Ac8 (16% of the control). Under all treatments, the highest stomatal conductance was shown in the morning between 6:45 - 9:10 am. In optimally watered plants, stomatal conductance was > 200 mmol $m^{-2}s^{-1}$ in the morning which was reduced continuously throughout the day to a value around 50 mmol $m^{-2}s^{-1}$ in the late afternoon (data not shown). However, in severely stressed plants, all values were below 150 mmol $m^{-2}s^{-1}$, which was further reduced in a similar pattern as irrigated plants.

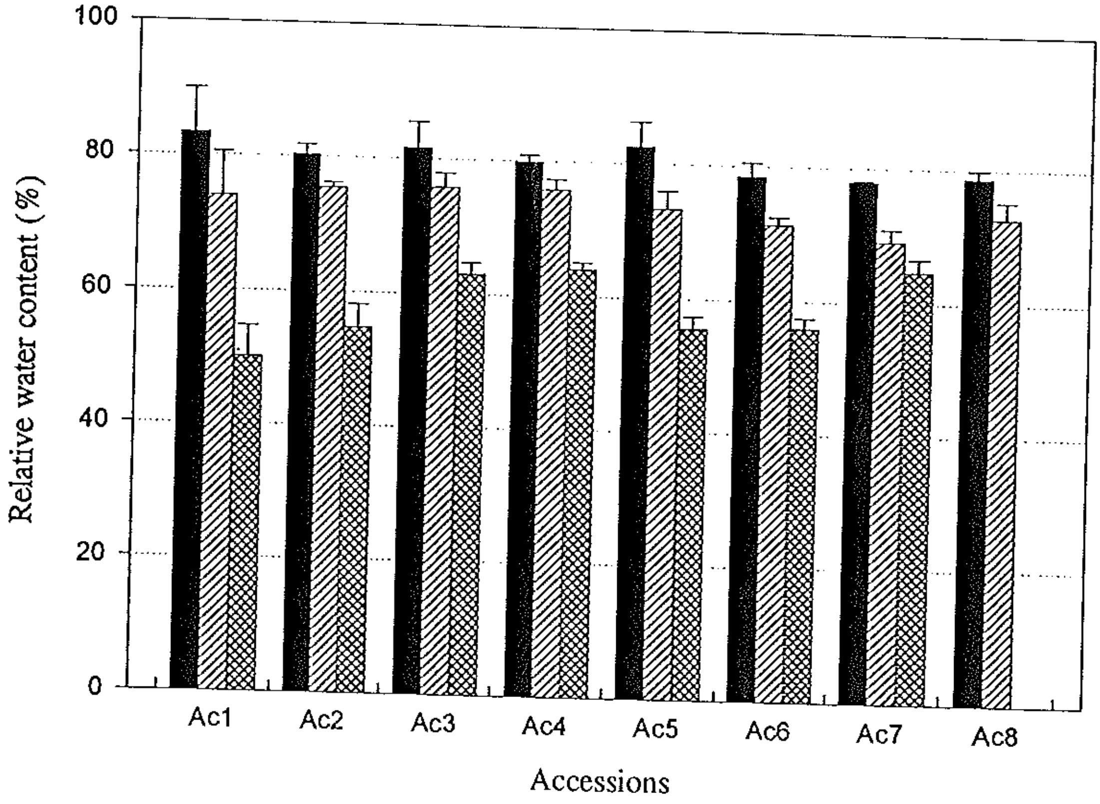


Fig. 9. Relative water content of leaf of chickpea accessions under three moisture regimes:control (■), moderate stress (▨) and severe stress (▩). Bars indicate S.E. of means (n=2-6).

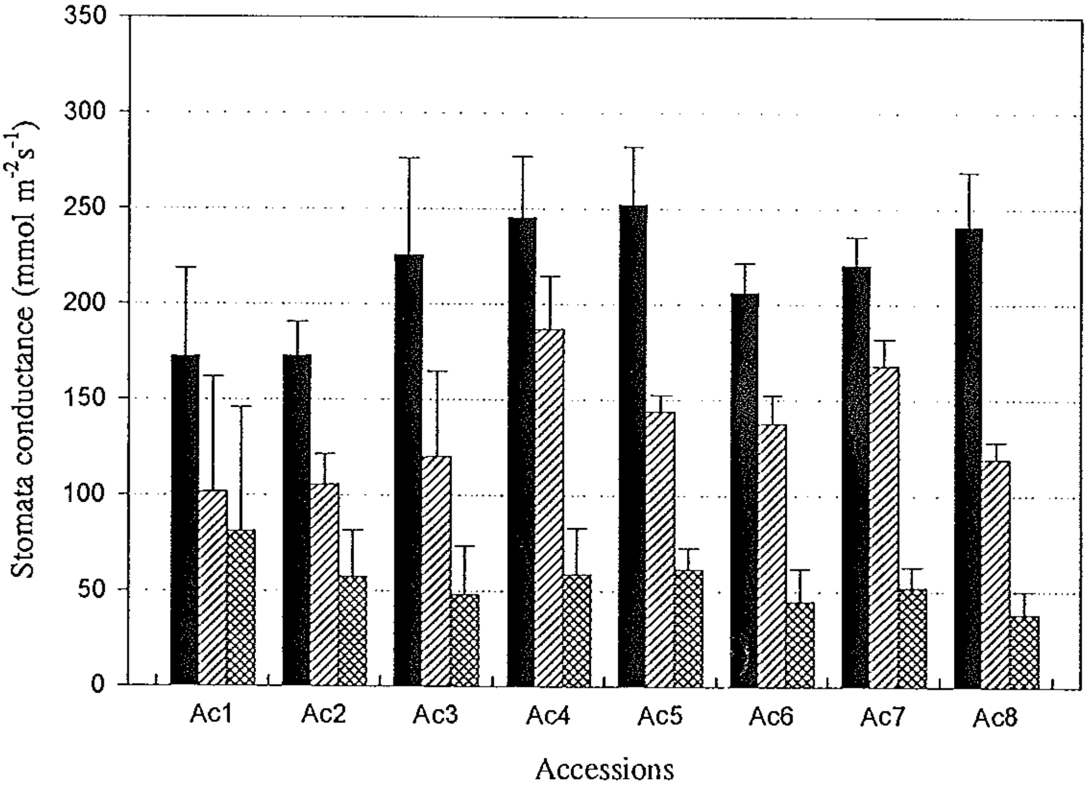


Fig. 10. Stomatal conductance of the abaxial leaf surfaces of eight chickpea accessions under three moisture regimes: control (■), moderate stress (▨) and severe stress (▩). Bars indicate S.E. of means (n=3-6).

3.3. Chlorophyll *a* fluorescence

The ratio of variable to maximum fluorescence was used to estimate maximal quantum yield of photosystem II (Fv/Fm). According to Björkman and Demming (1987) an Fv/Fm value of 0.83 is considered to be an average reference point for the optimal quantum efficiency of unstressed leaf of higher plants. Maximal photochemical efficiency (measured at midday) showed no variation either in optimally watered or stressed plants in all accessions studied (Fig. 11). The mean value of Fv/Fm at midday ranged from 0.633 to 0.821 regardless of watering regime. In addition, no significant differences among accessions was observed in all treatments. In both stressed and control plants, the ratio Fv/Fm displayed a decrease between 12:00 and 14:00 h (Fig. 12a). However this reduction was recovered at the end of the afternoon, which revealed that the plants were not irreversibly photoinhibited. Similarly, fluorescence parameter Fm (maximal fluorescence) showed also diurnal decrease that demonstrated thermal dissipation of excess excitation energy (Fig. 12b).

The ratio of Fv/Fm of dark adapted samples were also estimated using the mini-pam. However Only optimally and moderately stressed plants were used for the measurement. Similar results were obtained when the same was estimated using the PEA (data not shown). However accession Ac8 exhibited significantly lower value under moderately stressed levels than the control.

The effective quantum yield of photosystem II as measured by ΔF/Fm' tended to decline with moderate water stress treatment (Fig. 13). Percent reduction in ΔF/Fm'ranged from 16 to 33% in most accessions, however the lowering was statistically significant only in accessions Ac7 and Ac8. Among the accessions, Ac1 and Ac3 showed the least reduction in ΔF/Fm' due to water stress.

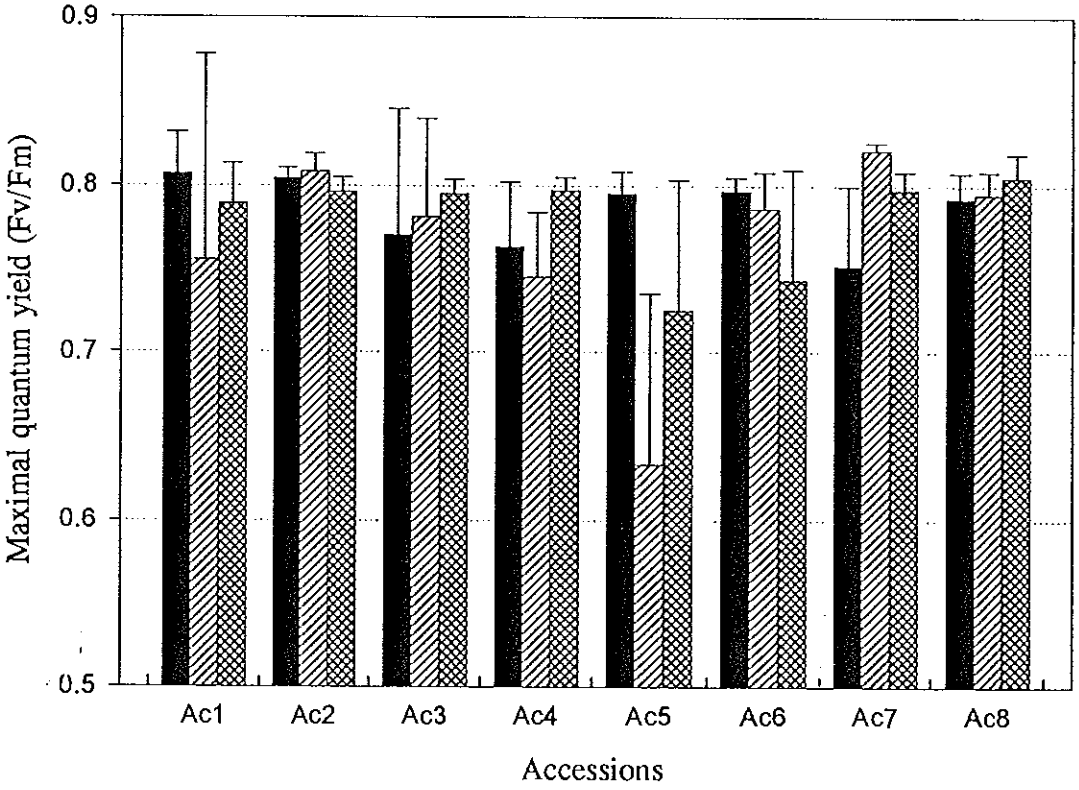


Fig. 11. Maximal quantum yield (Fv/Fm) in leaf of chickpea accessions measured by PEA under three moisture regimes: control (■), moderate stress (▨) and severe stress (▩). Bars indicate S.E. of means (n=2-6).

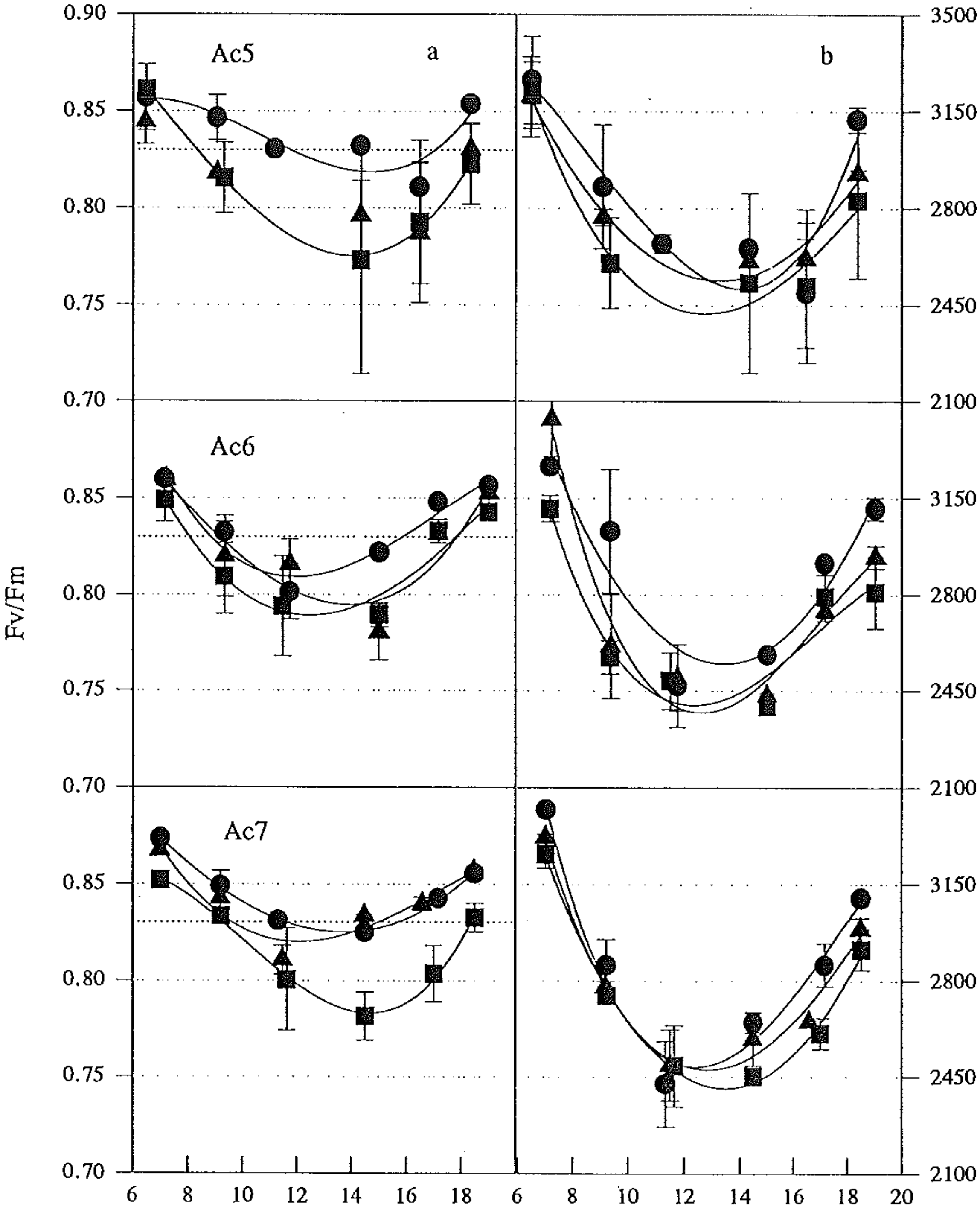


Fig. 12. Diurnal change in a) Fv/Fm and b) Fm of chickpea leaf under three moisture regimes: control (●), moderate stress (▲) and severe stress (■). Bars indicate S.E. of means (n=2). Data of three accessions are shown.

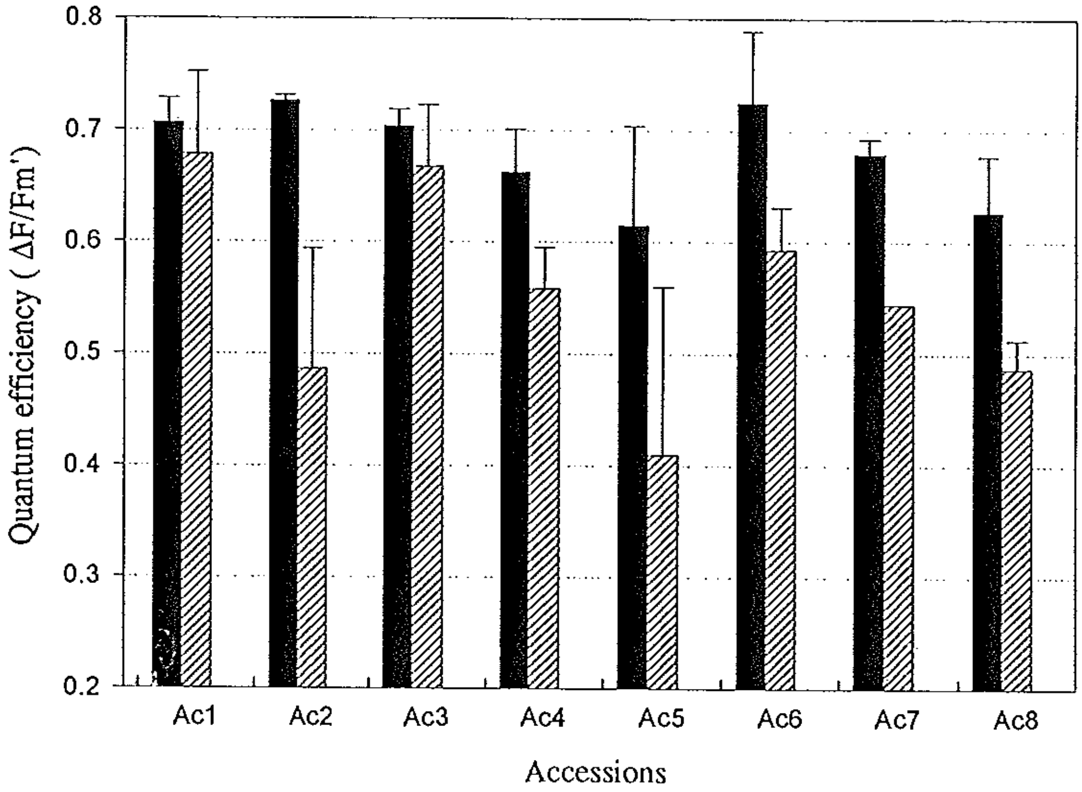


Fig. 13. Quantum efficiency of photosystem II (ΔF/Fm') of chickpea leaf under two moisture regimes: control (■) and moderate stress (▨). Bars indicate S.E. of means (n=2-6).

Light saturation curves for both optimally watered and moderately stressed plants were generated using mini-pam at different times of the day. However, only midday data is shown for comparison purposes. The resulting light response curves showed that water stress caused a substantial decrease in apparent electron transport rate (ETR) in accessions Ac5, Ac6, Ac7 and Ac8 with respect to control plants (Fig. 14). However, the other accessions exhibited more or less similar curves that were saturated at low photon flux density (PFD) and ETR. In well watered leaf ETR saturated at PFD greater that 2000 μmol photons $m^{-2}s^{-1}$ and rates of 240 - 250 μmol electrons $m^{-2}s^{-1}$ for Ac5, Ac6 and Ac8. But under moderately stressed plants, these values were lower (1200 - 1500 μmol photons $m^{-2}s^{-1}$ and 100 - 130 μmol electron $m^{-2}s^{-1}$, respectively). Among the accessions, Ac7 showed the lowest (50 - 60 μmol electrons $m^{-2}s^{-1}$) rates when subjected to moderate water stress. Similar trend were observed in the data for the effective quantum yield (Fig. 15). It is obvious that as PFD increase the values of effective quantum yield will decrease. However, in our experiment, the reduction was more pronounced in water stressed leaf of accessions Ac5, Ac6, Ac7 and Ac8.

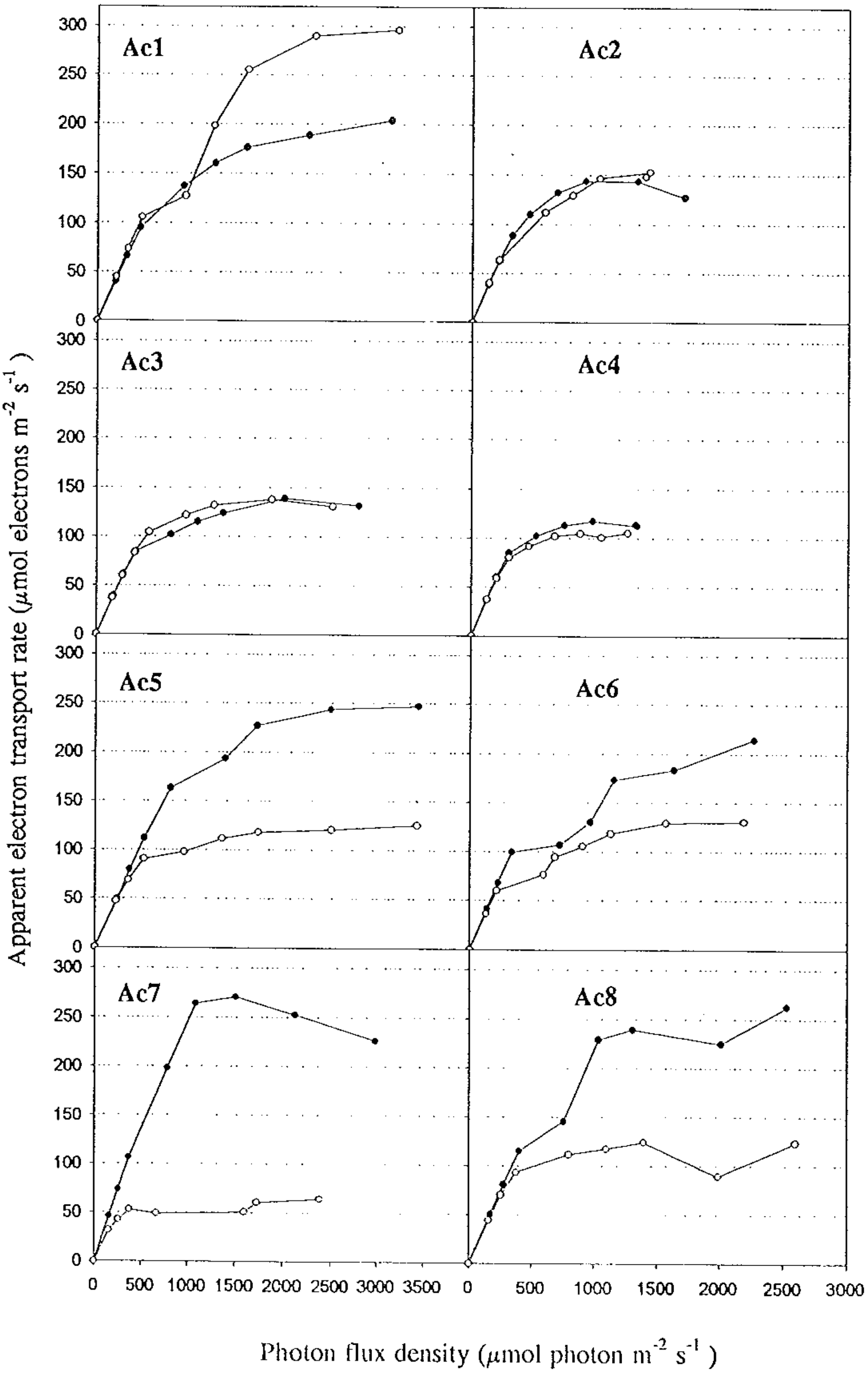


Fig. 14. Response of apparent electron transport rate to irradiance in chickpea leaf under two moisture regimes: control (—•—) and moderate stress (—○—).

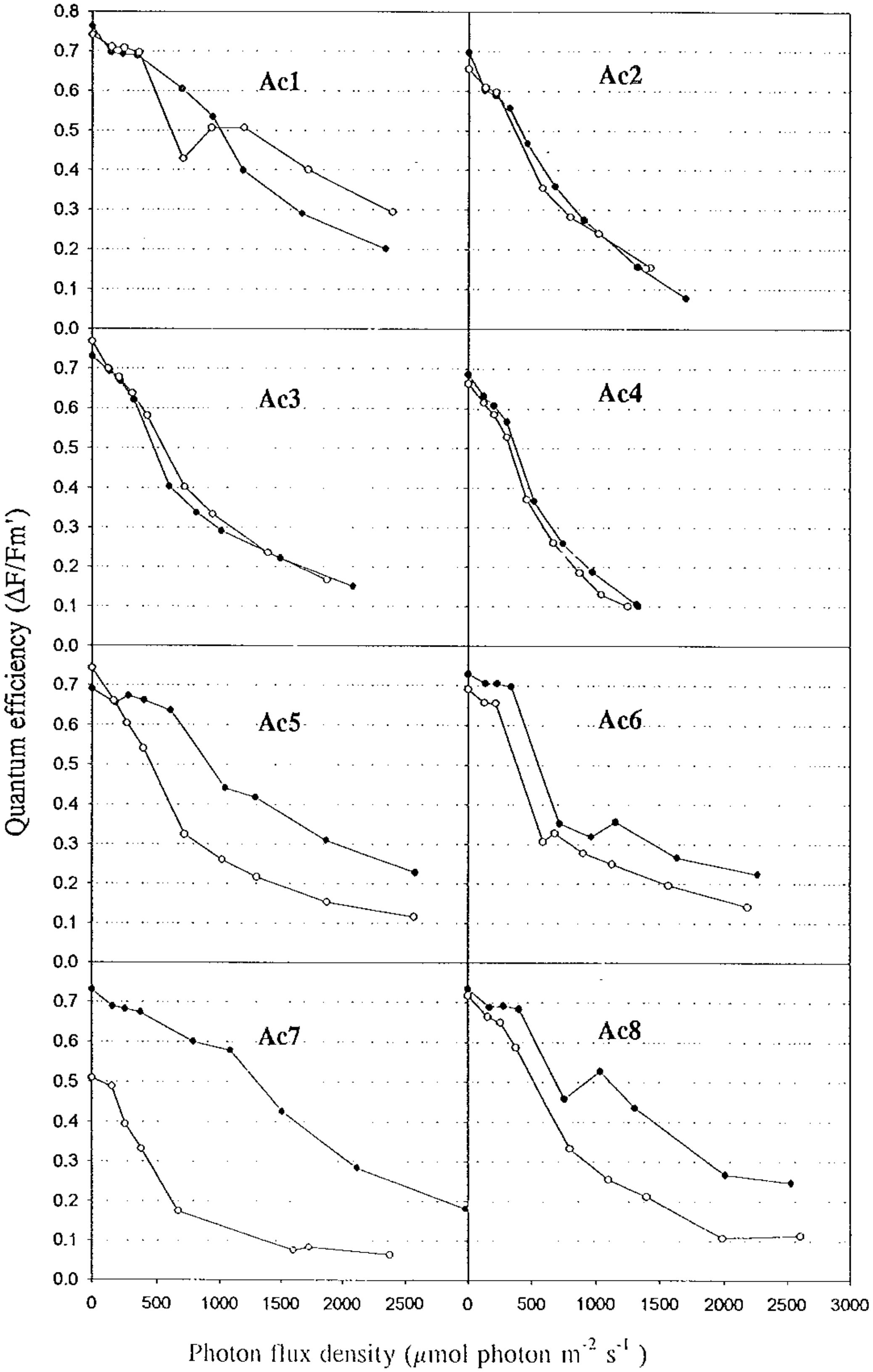


Fig. 15. Change in quantum efficiency (ΔF/Fm') with increasing irradiance in chickpea leaf under two moisture regimes: control (—•—) and moderate stress (—○—).

3.4. Cell membrane stability

By using PEG as a desiccant, variations among accessions in Relative Injury (RI) of the membrane was observed (Table 2). Accessions Ac4 and Ac5 showed the least tissue damage as measured by RI. In contrast, accessions Ac3 and Ac6 maintained the highest RI values, 80.21% and 86.66%, respectively.

Table 2. Mean percentage of tissue damage of eight chickpea accessions measured by using PEG as a desiccant and leaf from well-watered plants.

Accession	RI (%)
Ac1	76.71
Ac2	78.02
Ac3	80.21
Ac4	56.05
Ac5	51.46
Ac6	86.66
Ac7	62.10
Ac8	66.77

3.5. Seed yield and yield components

Water stress treatment significantly reduced seed weight per plant in all accessions (Fig. 16). There were also significant variations in seed weight between plants grown in moderate and severe stress levels. No marked differences were observed among accessions in all watering regimes. Similarly water stress affected dry weight of pods and number of pods per plant (Fig. 17). The mean number of seeds per pod ranged between 1.20 - 1.34, 1.14 - 1.35 and 1.0 - 1.34 in the control, moderately and severely stressed plants, respectively (Table 3).

Table 3. Mean number of seeds per pod of chickpea accessions under three watering regimes. (n=4-13, mean±sd).

Accessions	Control	Moderate stress	Severe stress
Acl	1.30 ± 0.10	1.35 ± 0.19	1.11 ± 0.25
Ac2	1.23 ± 0.11	1.14 ± 0.10	1.05 ± 0.18
Ac3	1.27 ± 0.18	1.22 ± 0.21	1.23 ± 0.37
Ac4	1.25 ± 0.10	1.26 ± 0.13	1.00 ± 0.00
Ac5	1.20 ± 0.13	1.18 ± 0.07	1.18 ± 0.32
Ac6	1.22 ± 0.09	1.20 ± 0.12	1.11 ± 0.23
Ac7	1.24 ± 0.14	1.16 ± 0.14	1.08 ± 0.20
Ac8	1.34 ± 0.18	1.26 ± 0.13	1.34 ± 0.45

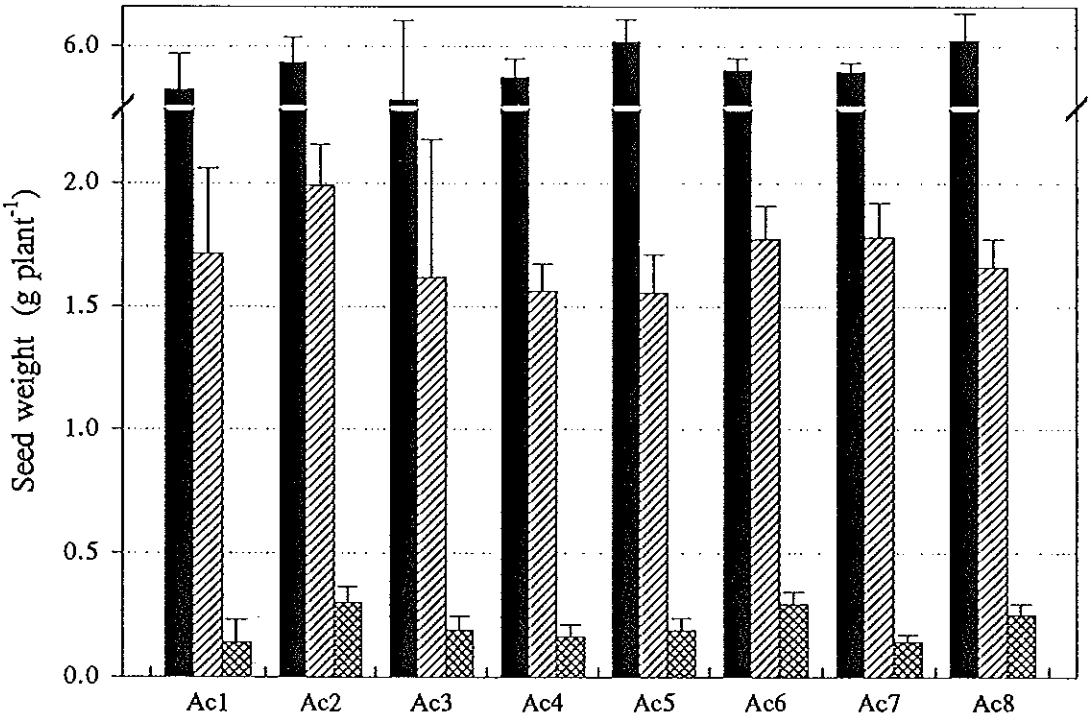


Fig. 16. Seed weight of eight chickpea accessions under three moisture regimes: control (■), moderate stress (▨) and severe stress (▩). Bars indicate S.E. of means (n=4-13).

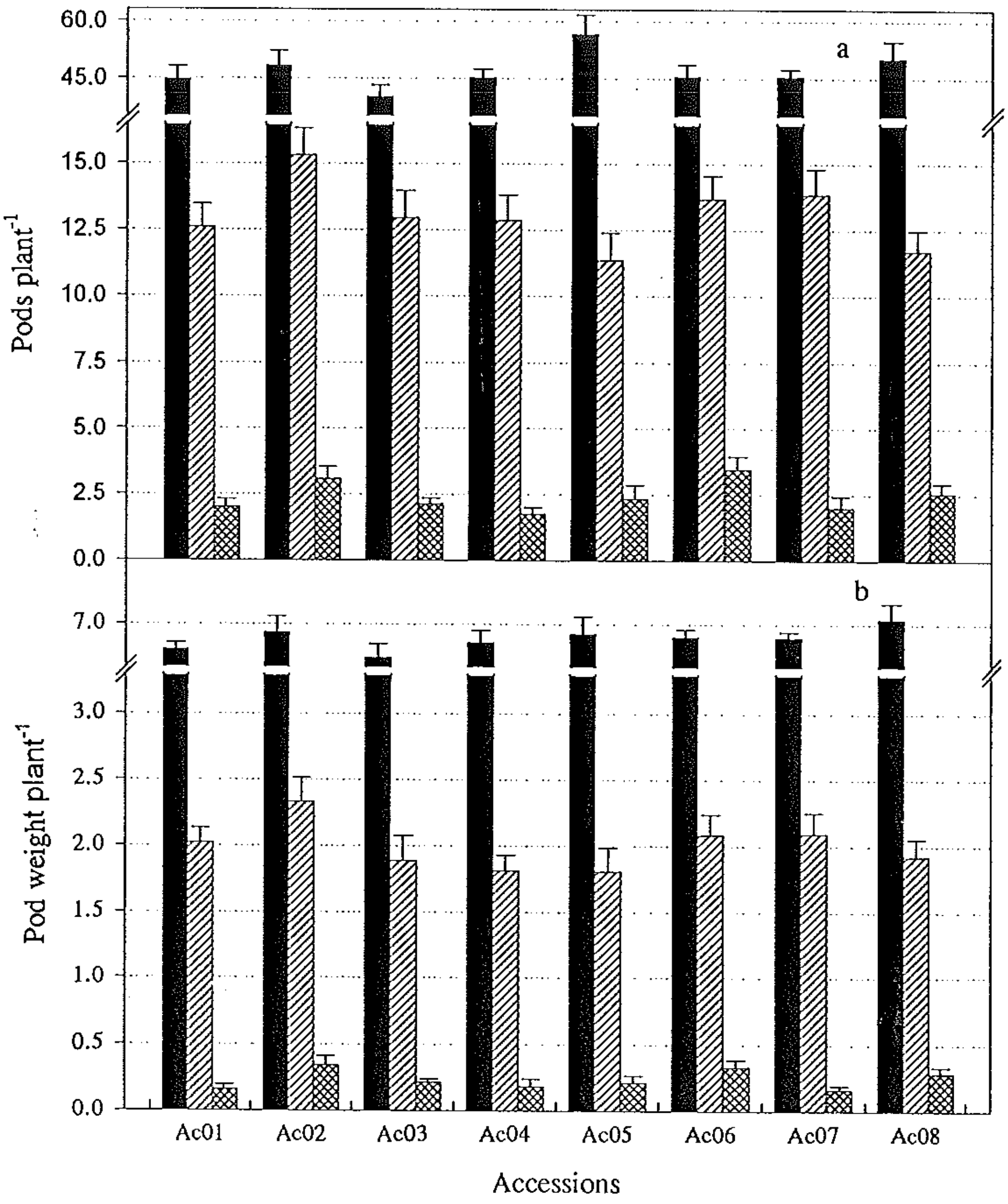


Fig. 17. Effect of water stress on yield components a) number of pods per plant and b) pod weight per plant of chickpea accessions under three moisture regimes: control (■), moderate stress (▨) and severe stress (▩). Bars indicate S.E. of means (n=4-13).

4. DISCUSSION

4.1. Plant growth

In the present study, water stress in chickpea significantly affected vegetative growth including biomass production, plant height and total leaf area. Our result is in agreement with the findings of Masresha Fetene (1985) for cowpea, Tilahun Amde (1998) for faba bean, pea, and chickpea, Singh et al.(1997) for chickpea and Pelleschi et al. (1997) for maize. In general, reduction in biomass production may be attributed to reduction in relative water content that affects various physiological processes including photosynthesis, enzyme activity and metabolism (Singh et al., 1997). According to Singh and Srirama (1989) and Thomas and Fukai (1995), decline in biomass production as result of water deficit in chickpea was associated with a greater reduction in radiation interception particularly due to leaf senescence. Their results support our observation that limited water supply accelerated senescence of older leaf in comparison to control plants.

Plant height was found to be sensitive to water stress during the vegetative growth. Reduction caused by water stress in plant height was also reported in maize by Pelleschi et al. (1997) in cowpea by Masresha Fetene (1985). Furthermore, the effect become more pronounced between treatments as the duration of drought progressed. This result is in consistence with the observation of Nielson and Nelson (1998) in bean plants.

Total leaf area declined significantly when chickpea plants were exposed to water stress, which is in agreement with the observation of Singh (1991) and Thomas and Fukai (1995). Furthermore, this reduction was strongly correlated with decrease in leaf number as observed in stressed plants. This result agrees with those found by Pankovic et al. (1999) using sunflower. These authors reported that both total leaf area and number of leaf were lower in water stressed sunflower. But our result contrasts with the observation of Centritto et al. (1999) that drought reduced leaf area without affecting the leaf number in cherry. This may be due to the effect of water stress on cell division and cell enlargement that are the main component of cell growth and leaf expansion (Kramer, 1983). Thus, small water deficit will result in a reduction in cell volume and a marked decline in leaf area. For instance, Schmidhalter et al., (1998) reported that the elongation of maize leaf was highly sensitive to slight reduction in soil water content and completely stopped at severe stress. However, in our experiment, further leaf area reduction was caused by enhanced abscission that have also affected the total number of leaf. This is supported by observation of Delgado et al. (1992) in tobacco, whereby change in mean leaf size and number of leaf per plant were considered to be the reason for reduced leaf area in stressed plants.

In general, well watered plants maintained higher leaf area, however, both severely stressed and moderately stressed plants reduced their area in order to minimize water loss by transpiration since water use efficiency (WUE) in chickpea is reported to be lower than in other crop plants, for instance barley (Sivakumar and Singh, 1987; Thomas and Fukai, 1995). This phenomenon may be considered as a drought avoidance mechanism.

Our results confirm the report of Tilahun Amde (1998) that both shoot and root growth rate in chickpea are reduced by water stress, which was clearly reflected in the reduced biomass under stress conditions. Similar result was obtained in cowpea (Masresha Fetene, 1985) and *Eucalyptus* (Roden and Ball, 1996). Reduced growth rate of stressed plants may be explained by greater reduction in leaf area which in turn affects photosynthesis. In general, all accessions responded to water stress in similar way when RGR is considered.

In the present study, Root/shoot ratio remained unchanged under all watering regimes except in accession Ac5. However, under severe stress condition the ratio in accession Ac5 was found to decrease. This is in agreement with the findings of Busso et al. (1998) in *Medicago* and *Erodium spp.* According to Sobrado and Turner (1986) cited in Busso et al. 1998, similar root/shoot ratio in both stressed and well watered plants may be attributed to similar degree of osmotic adjustment both in root and leaf cells. But our result is in contrast with the observation of increased root/shoot ratio under stress in several crop plants for instance, in cowpea (Masresha Fetene, 1985) and maize (Pelleschi et al., 1997; Schmidhalter et al., 1998). Similarly, accession Ac7 showed an increasing trend with stress although not significantly. This discrepancy may be partly attributed in our case to use of smaller pots (30 x 20 cm) that may have limited root growth. Thus, further investigation is necessary by employing bigger pots and conducting field experiments before a generalized conclusion is reached.

Biomass allocation to different organs was similar in well watered control and stressed plants. Between 40 and 50% of the biomass was allocated to leaf followed by 20 - 30% to stem and roots and 0 - 20% to pods. This result confirms the findings of Singh (1991) however, this author reported that water deficit had no influence on biomass allocation to leaf and branches prior to pod initiation. During the reproductive phase, water stress increased allocation to pods. In the present study, biomass allocation data were collected at 57 DAP while the plants had started to initiate pods. Furthermore, as the stress level increased, most plants showed an increasing pattern of allocation to their reproductive organs. Thus, perhaps one may need to wait for the pods to develop in order to see the effect. However, this result does not agree with the reports of decrease in partitioning of total plant biomass to leaf and stem in *Medicago* and *Erodium spp.* (Busso et al., 1998). This may be explained by the indeterminate growth of chickpea where vegetative growth continues during reproductive phase, thus competing for assimilate with the reproductive organs (Singh, 1991). Significant differences were observed among accessions under moderate stress conditions. Thus, biomass partitioning might be a useful tool to identify susceptibility to drought together with other parameters.

4.2. Water relation

The water status of a plant can best be explained in terms of its RWC and water potential. In the present study, RWC was lowered due to drought stress reaching 50 - 60% compared to 80 - 85% in well-watered plants. This result is in agreement with the findings of Tilahun Amde (1998) who reported an RWC value of about 80% in optimally watered plants. The water loss in stressed plants was associated with a decrease in water potential. In the present study, leaf water potential reached -2.0 to -2.5 MPa under severely stressed conditions. The reduction in leaf water potential due to water deficit can be explained by a decrease in absorption and translocation of water as the result of loss of gradients in water potential between the soil and roots as well as decline in transpiration pull (Singh et al., 1997).

Although osmotic potential was not determined in this study, Tilahun Amde (1998) reported reduction in this component of water potential in chickpea. This author concluded that chickpea tolerates water deficit with higher osmotic adjustment and lowered water potential. Under severe stress condition, RWC varied among accessions thus, it can be used (together with leaf water potential) as a simple tool for screening drought tolerant chickpea varieties.

For instance, EL Hafid et al. (1998) reported that in drought susceptible wheat genotypes, water content showed a greater decline to reach lower water potentials than those of tolerant cultivars.

Comparison of stomatal conductance in well-watered and stressed conditions revealed that optimally watered plants showed high conductance than stressed plants. Similar results were reported in cowpea (Masresha Fetene, 1985), bean (Castonguay and Markhart, 1992), tomato (Biehler et al., 1997), soybean (De Souza, et al., 1997), maize (Pelleschi et al., 1997), clover (Socias et al., 1997) and sunflower (Pankovic et al., 1999). On diurnal basis, the highest stomatal conductance was shown in the morning for all treatments, thereafter it decreased rapidly at noon for moderately and severely stressed plants. However, the reduction was gradual in well watered plants. Similar observation was made by Delgado et al. (1992), Socias et al. (1997) and Ekanayake and De Jong (1992). Thus, the present study confirms the finding that stomatal conductance depends on the availability of water in the soil. Furthermore, there is sufficient evidence to show that ABA is produced by the root due to water stress and transported to the leaf through the transpiration stream to cause stomatal closure (Lösch and Schulze, 1995).

On the other hand, our result disagrees with the findings of Tilahun Amede (1998) who reported a stomatal conductance value of between 80 - 120 mmol $m^{-2}s^{-1}$ under drought condition. Perhaps this might be attributed to higher vapour pressure deficit in our glasshouse that caused stomata to respond differently since the sensitivity of stomata to humidity is well documented in the literature. Thus, by taking only the morning measurements (6:45 - 9:10 am), the present study showed significant differences among accessions under moderate stress conditions. This variation may allow differentiation of genotypes in terms of tolerance or susceptibility. Furthermore it has the advantages of being very sensitive as it shows variation before higher reduction in water potential. Also it is established that drought tolerant varieties maintain higher conductances than susceptible one's under severe water stress condition. El Hafid et al. (1998), for instance, demonstrated that drought tolerant wheat genotypes exhibited a higher stomatal conductance than susceptible genotypes under severe stress. Similar observations were also made by Ramanjulu et al. (1998).

4.3. Chlorophyll *a* fluorescence

As indicated by Björkman and Poweles (1984), survival of plants during long-term drought conditions require prevention of photoinhibition. In the present study, the potential photoprotective response of chickpea leaf due to water stress have been investigated. Maximal photochemical yield (Fv/Fm) measured both by Plant Efficiency Analyzer and a pulse-amplitude fluorometer showed no significant differences between optimally watered and drought stressed plants. Our result supports the findings of Cechin (1998) in sorghum, Flagella et al. (1998) in wheat and Pankovic et al. (1999) in sunflower. Calatayud et al. (1997) suggested that PSII was found to be extremely resistant to drought in the dark in contrast to heat and light stress. In addition, when the diurnal course of this parameter is considered, there was a decline between 12:00 and 14:00 h both in the control and stressed plants, which recovered later in the afternoon. Similar observations were made on leaf of oak trees (Epron et al., 1992) and olive trees (Angelopouls et al., 1996) subjected to water stress. In the present experiment a decrease in initial (Fo) and maximum (Fm) fluorescence parameters were observed. This reduction may probably reveal the onset of mechanisms of thermal de-excitation (Epron et al., 1992). Dissipation of excess energy in the form of heat has been reported to be efficient in preventing damage to PSII reaction centers (Epron et al., 1992; Cechin, 1998). Result of the non-photochemical quenching of fluorescence of both stressed and non-stressed leaf, although scattered (data not shown) appeared to support the above observation for few accessions because the measurement was affected due to cloudiness of the day as previously reported by Schindler et al. (1996). No differences were observed in maximal photochemical yield among accessions as previously observed by Flagella et al (1998). Thus, Maximal photochemical yield may not be recommended for use as a selection tool.

On the other hand, quantum efficiency (ΔF/Fm') measured at ambient conditions was found to decline as the result of drought stress. Similar reports were found by a number of workers who investigated the effect of water stress on the actual quantum yield (Flagella et al., 1995; 1996; 1998; Cechin, 1998). This observation is further supported by gradual lowering of ΔF/Fm' with increasing irradiance indicating that most of the primary quinone acceptors were in reduced state. Quantum efficiency was also strongly associated with a reduction in photosynthetic ETR as previously reported by Masresha Fetene et al. (1997) and Basu et al. (1998). However, water stressed leaf showed relatively lower values compared to the controls for instance, in accessions Ac5, Ac6, Ac7 and Ac8. Basu et al. (1998) suggested that ΔF/Fm' may decrease if reoxidation of QA in light is inhibited by partial blockage of electron transport from PSII to PSI by stress factors such as drought.

As shown by others (Flagella et al., 1995; 1996; 1998) differences in quantum efficiency was observed among accessions when subjected to water stress. Furthermore, Flagella et al. 1996 demonstrated that susceptible wheat cultivars showed higher percent decrease in quantum efficiency than tolerant cultivars. In addition percentage decrease in ΔF/Fm' was found to correlate with drought susceptibility index of the cultivar in the field (Flagella et al., 1995). Therefore, Flagella et al. (1996) suggested that maintenance of quantum efficiency may be considered as a good indicator of drought tolerance. Taking the observed variations in quantum efficiency in chickpea accessions due to water stress, this parameter could be used for screening tolerant varieties. However, this should be further evaluated in the field using known tolerant and susceptible cultivars before a generalized conclusion is reached.

Considerable variation was observed in cell membrane stability among chickpea accessions. Similarly Belay shiferaw and Baker (1996) observed cultivar differences in terms of tissue damage as the result of desiccation in *tef*. According to Levitt (1980) membrane dysfunction is one of the main physiological processes of plant cells disturbed by water stress that lead to increased electrolyte leakage. Perhaps it might be due to dehydration strain leading to mechanical stress on membrane proteins (Levitt, 1980). Thus, by quantifying electrolyte leakage, it might be possible to evaluate drought tolerance in chickpea.

4.4. Yield and yield components

It is apparent from the responses observed for yield and yield components that productivity in chickpea varies with irrigation. By comparing seed weight in irrigated and non-irrigated treatments, water stress resulted in significant decrease in seed weight. Reductions caused by water deficit in seed weight was also reported in cowpea (Masresha Fetene, 1998), chickpea (Thomas and Fukai, 1995), bean (Nielson and Nelson, 1998; Singh, 1995) and soybean (De Souza et al., 1997). In general, seed weight reductions were associated with decline in number of pods per plant. This observation supports previous reports that water stress reduced seed weight through a reduction in branch number and pod abortion resulting in poor pod set (Sivakumar and Singh, 1987; Thomas and Fukai, 1995). This may be partly attributed to the fact that water stress during flowering stage affects anther development and meiosis of gametes (Henckel, 1964).

Number of seeds per pod was not significantly affected by drought as previously observed by Nielson and Nelson (1998). In addition, although seed size was not determined, we observed that seeds from severely stressed plants were smaller. It seems plausible that seed weight declined due to reduction in allocation of C and N due to reduced N_2-fixation and photosynthesis (Hooda et al., 1989). As has been mentioned earlier, water stress may affect photosynthesis through stomatal limitation and premature senescence of leaf that limited the source. Although no variation among accessions were found in seed weight, this parameter should be considered during screening for tolerance because selecting high yielding varieties is the ultimate objective of any research in drought prone regions.

5. SUMMARY AND RECOMMENDATIONS

The present study attempted to investigate the effect of water stress on growth and physiology of eight chickpea accessions. The results suggested that all growth parameters studied were affected by water stress contributing to lower biomass in stressed plants. However, no genotypic variations were observed on these parameters. Thus, these parameters were not found to be suitable for selecting drought tolerant varieties. Chickpea plants subjected to water stress reduced water loss through reduction in leaf area and by maintaining lower stomatal conductance. The negative correlation noted in stomatal conductance with drought stress and the presence of differences among chickpea accessions on the same deserve further studies in the field. Stomatal conductance together with RWC appeared to be good candidates as selection tools.

It was observed that soil water depletion induced a reduction in quantum efficiency (ΔF/Fm') and ETR. The reduction in quantum efficiency was accompanied by enhancement of thermal dissipation of excess energy which was indicated by a decline in Fm. This mechanism allows an efficient protection of PSII before permanent damage occurres. Moreover, differences among accessions were demonstrated that indicated the potential use of this parameter for screening purposes. Nevertheless, further studies relating quantum efficiency with degree of drought tolerance need to be conducted under field condition.

The present study has shown the existence of variations in tolerance to membrane damage among chickpea accessions due to desiccation. Although seed weight declined in stressed plants, stressed plants tended to allocate more assimilates towards their pods compared to optimally watered plants. This characteristics may be taken as a drought avoidance mechanism.

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