Indian J. Plant Physiol., Vol. 15, No. 2, (N.S.) pp. 117-124 (April-June, 2010)

LEAF GAS EXCHANGE, CHLOROPHYLL FLUORESCENCE, GROWTH AND ROOT YIELD OF ASHWAGANDHA (*WITHANIA SOMNIFERA* DUNAL.) UNDER SOIL MOISTURE STRESS

SONAL SHAH, R. SARAVANAN* AND N.A. GAJBHIYE

Directorate of Medicinal and Aromatic Plants Research, Boriavi (PO), Anand, Gujarat-387 310, India

Received on 3 August, 2009, Revised on 01 June, 2010

SUMMARY

Aswagandha (*Withania somnifera*, Dunal.) is an important medicinal plant. It is cultivated in India as rainfed crop for its roots. A field study was conducted to understand the response of this crop (cv. JA-134) to progressive soil moisture deficit. The treatments imposed were moderate (2 irrigations) and severe stress (single irrigation) along with well watered control (4 irrigations). As the soil moisture stress progressed during the crop growth period, $ø_{soil}$ decreased in stress treatments and reached to -10.93, -1.15 MPa at 169 DAS in severe stress, moderate stress at 30 cm soil depth whereas control had $ø_{soil}$ of -0.0088 MPa. Correspondingly predawn leaf water potentials were -0.615 and -0.506 MPa in severe and moderate stress treatments while, control sustained the ø of -0.373 MPa. At 169 DAS, total chlorophyll content was reduced 49% and 60% in moderate and severe stress, whereas proline content increased 2.75 and 3.96 times that of control in moderate and severe stress. Gas exchange and chl-a fluorescence were significantly altered under stress. Moisture stress reduced all the growth parameters studied compared to control plants. Reduction in dry weight of leaves and stems were 19.88% (3.52 g), 36.48% (5.19 g) respectively in severe stress compared to control. Whereas root dry weight increased 35% and 20% respectively in moderate and severe stress compared to control. The increased root biomass partitioning and higher root yield under soil moisture stress helped in off-setting the deleterious effect of water stress.

Key words: **Aswagandha, chlorophyll, fluorescence, gas exchange, moisture stress**

INTRODUCTION

Ashwagandha *(Withania somnifera* Dunal*)* also known as Indian Ginseng is widely used in Indian system of medicines including Ayurveda and it is an ingredient of many herbal formulations prescribed for musculoskeletal conditions. It is grown as late rainy season (kharif) crop in the semi-arid regions of Western and Central India receiving 500 to 750 mm rainfall. It is cultivated with residual moisture in soil in addition to single or two irrigations during the entire period of its growing season. The crop responds well if one or two winter rains are received during its early growth stages, with good plant growth and enhanced root yield. Although *W. somnifera* perform well in relatively dry regions with limited soil moisture during its growing period, prolonged soil moisture deficit inhibits the growth and development of the crop resulting in decreased yield and plant productivity. This crop matures at 150-180 days after sowing (DAS) which makes the crop prone to severe soil moisture stress during late growth stages. Incidentally the seed maturation and root bulking occurs

*Corresponding author, E-mail: rajusar@gmail.com

after 100 DAS which corresponds to lower soil moisture and high solar irradiance. Plants respond to drought through morphological adaptations (e.g. tapping ground water by deep roots and closing stomata to reduce water loss) and by series of physiological and biochemical mechanisms to minimize stress (Yamaguchi-Shinozaki and Shinozaki 2006). These internal responses range from changes in photosynthetic activity to the development of antioxidant defenses to enhance drought tolerance. Depending on the stress conditions (timing and intensity) of the target environments, some adaptive traits can be considered for yield improvement under drought if they enable plants to cope with a stress event that tends to occur every year at the same growth stage. Late season soil moisture deficit influences plant growth and root yield and hence a study was undertaken to characterize the response of *W. somnifera* to prolonged soil moisture deficit during late growth stages and to understand physiological processes and adoptive mechanisms which operate to counter the effects decreasing soil moisture and high solar irradiance.

MATERIALS AND METHODS

The study was carried out at Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat, India. The average rain fall is 800 mm and temperature (max – min) ranged between 42 °C and 12.7 °C. The soil is sandy loam and had pH 7-7.5 with water holding of 20-22%. The study was conducted during winter and spring season between Oct. 2008 and Apr. 2009. The design was RBD with three treatments (well watered - control, mild water stress and severe water stress) with 7 replications each. Seeds of cv JA 134 (*Withania somnifera* (L.) Dunal) were sown in lines with 30 cm line spacing on 15th October 2008 and standard cultural practices were carried through out the crop growth period. The severe water stress treatment received one flood irrigation (67 DAS-Days after sowing, i.e 22nd Dec., 2008) and mild stress had two irrigations (67 DAS on 22nd Dec. 2008 & 114 DAS on 7th Feb. 2009), whereas control treatment received four irrigation (67 DAS on 22nd Dec. 2008, 114 DAS on 7th Feb, 153 DAS on 18th March and 168 DAS on 2nd April 2009). Data on soil water potential (Ψ_{soil}), leaf water potential (Ψ) growth, gas exchange and other parameters were recorded on 77, 93, 110, 138, 152 and 169 DAS.

The water potential of soil was measured based on moisture release curve developed for a range of soil moisture content and resultant Ψ_{soil} using soil chamber psychrometer (Wescor Inc, USA) following Wacker, (1999). At each sampling date, leaf water potential (Ψ) of fully expanded leaf was measured with a pressure chamber (3005 Pressure Extractor, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) at pre-dawn. The leaf was inserted inside the pressure chamber in such a way that ~3-5 cm of the petiole remains outside and slowly air pressure was increased with nitrogen gas flow. Pressure at which xylem sap flow was initiated at the cut end was noted as Ψ of leaf sample.

Gas exchange parameters were measured simultaneously during fluorescence observations from the light adapted leaves using an open type infrared gas analyser (LI-6400, LI-COR Inc., Lincoln, NE, USA) attached with an integrated fluorescence chamber head (LI-6400-40 leaf chamber fluorometer, LI-COR Inc.) at ambient CO_2 and relative humidity during 10.30–12.30 h. Chlorophyll *a* fluorescence kinetics was measured on the adaxial surface of intact fully expanded leaf. Selected leaves were dark adapted for 1 hr before recording the fluorescence parameters (F_o, F_m). Immediately after that, other fluorescence and gas exchange parameters were recorded from light adapted leaf following manufacturer's manual. The minimal fluorescence (F_o) was measured with sufficiently low intensity modulated light (intensity setting 1 of the instrument). F_m was determined by a short (0.8 s) saturating pulse (light intensity setting 7). Steady–state value of fluorescence (F_s) was recorded at 1400 ì mol m^{-2} s^{-1} white actinic light. F_o2 was recorded just after darkening the light adapted leaf. A second saturating pulse similar to measuring F_m was applied to record maximal fluorescence in the light adapted leaf (F_m2).

The chlorophyll content from the leaves of *W. somnifera* was analyzed by the method described by Arnon (1949). The proline content from leaves was analyzed by the method using sulpho-salicylic acid and acid ninhydrin (Bates *et al.* 1973).

Data were analyzed by analysis of variance (ANOVA) with the help of MSTAT C programme to detect significant difference between means. The values

are mean ± SD for seven samples in each group. p values * 0.05 were considered as significant.

RESULTS AND DISCUSSION

Soil water potential ($Ø_{soil}$): Soil water potential in control, mild stress and severe stress treatments were shown in (Fig. 1-A). The $Ø_{soil}$ was maintained in a narrow range (-1.0 MPa) at 30 cm depth in both control and mild stress conditions. In severe stress treatment which received only one irrigation at 67 days after sowing and life irrigation, $Ø_{soil}$ did not vary significantly during the initial stages soil moisture depletion. However, $Ø_{soil}$ showed gradual reduction from -1.22 MPa at 125 DAS to -10.93 MPa at 169 DAS. The $Ø_{soil}$ did not vary during the period upto 125 DAS and it took another 56 days to reach this extreme soil water potential value. At this water potentials the plants could not extract any water at this depth of 30 cm top soil and all the water which the plant could obtain is from much deeper layers of the soil. Even though the $Ø_{soil}$ reached values near to -2.0 MPa at 125 DAS in the severe stress, it did not translate into reduced Ø probably due to the available moisture in the deeper layers of the soil. Plants responded to decreasing soil moisture stress at the early stage of stress signals by increasing the altering root biomass accumulation and by promoting the root growth which in turn help the plant to explore deeper soils for available water.

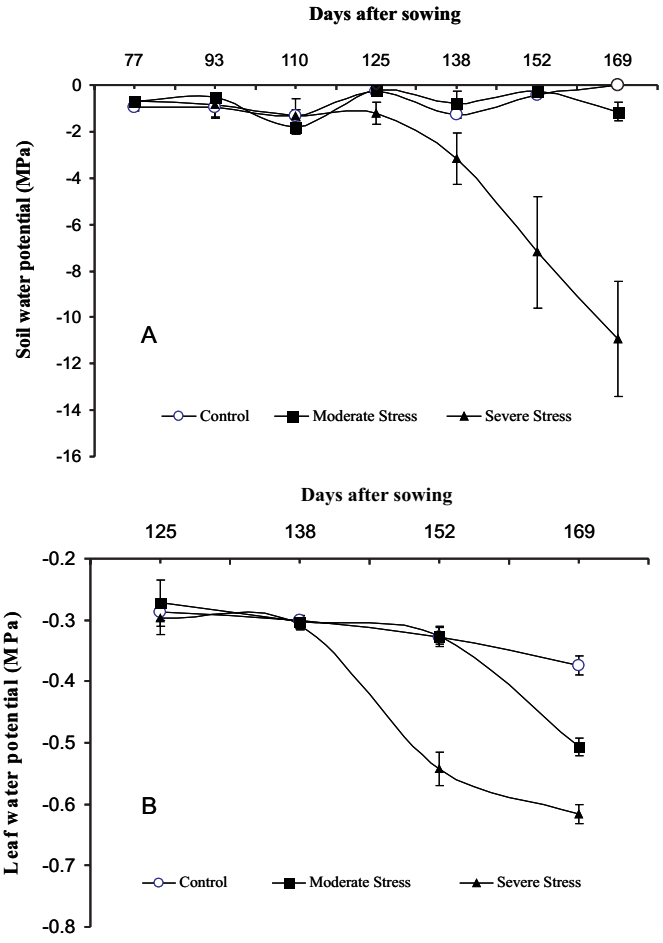

Fig. 1. Soil water potential (A) and leaf water potential (B) of *W. somnifera* plants grown under different irrigation regimes. The error bars represent SD (P=0.05).

Leaf water potential (Ø): Pre-dawn leaf water potential presented in Fig. 1-B was a good indicator of plant water stress which remained constant in the control plants during the period of study. Predawn leaf water potential (Ø) decreased in the mild and severe stress plants during the course of the water stress treatment period. After 125 DAS, predawn potentials were -0.29, -0.27 and -0.30 MPa for the control, moderate and severe water stress plants, respectively (Fig 1-B). However, the Ø started declining in the severe stress and reached -0.62 MPa at 169 DAS whereas, the control and mild stress had -0.37 and -0.51 MPa, respectively. Severe water stressed plants showed a difference of -0.24 MPa which is well in agreement with the loss of soil moisture and due to the extreme $Ø_{soil}$ recorded in this treatment.

Chlorophyll and proline content: Total chlorophyll content (TCC) in the control leaves were the highest and reached maximum (1.41 mg g^{-1} fr. wt. of tissue) at 125 DAS and decreased gradually afterwards until 152 DAS and later it peaked to 1.62 mg g^{-1} fr wt. at 169 DAS. Under moderate stress, TCC showed a decreasing trend from 1.16 mg g^{-1} fr. wt. of tissue (FWT) at 110 DAS to 0.82 mg g^{-1} FWT at 169 DAS. In severe water plants, there was a significant increase in TCC at 93 DAS reaching the maximum value of 1.26 mg g^{-1} FWT and drastically reduced with increasing moisture stress. At the end of the treatment period, plants had of TCC 0.823 and 0.643 mg g^{-1} FWT under moderate and severe water stress, respectively. Content of photosynthetic pigments was reduced significantly in plants experiencing drought stress. Several studies show that severe

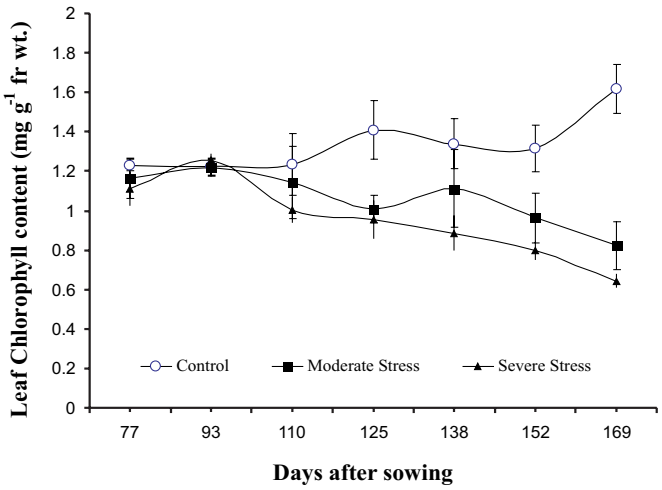

Fig. 2. Changes in leaf total chlorophyll content of *W. somnifera* plants grown under different irrigation regimes. The error bars indicate the SD (P=0.05).

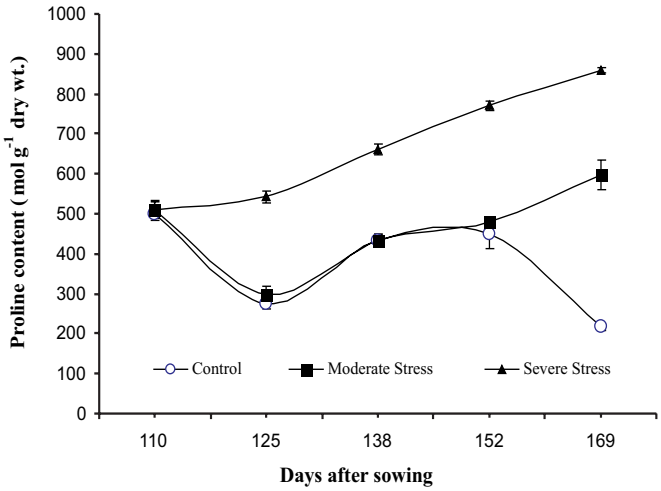

Fig. 3. Changes in leaf proline content of *W. somnifera* plants grown under different irrigation regimes. The error bars indicate the SD (P=0.05)

drought stress decreased the levels of chlorophyll *a*, *b* and total chlorophyll. The decrease in chlorophyll was attributed to the inhibition of chlorophyll synthesis as well as to accelerated turnover of chlorophyll already present. Soil moisture stress aggravates the damage caused to physiological processes along with increased canopy temperature and saturating light intensity. The loss of membrane integrity, turgor pressure loss in the cells, increased free radicals accumulation in cells eventually cause damage to cellular components and photosynthetic machinery. A reduction in chlorophyll levels might be the result of water stress or due to the chl a/b protein in drought-stressed plants (Alberte and Thornber, 1977).

Increased proline accumulation was recorded in stressed plants of *W. somnifera* under soil moisture stress (Fig. 3). At 169 DAS, moderate and severe stress had 2.75 and 3.96 times that of control. With the slow onset of stress in the field conditions, osmotic adjustment plays major role for maintaining the growth. In cultured cells, Handa *et al*. (1983) found that correlation coefficient for proline was low (0.51) before adaptation but increased significantly after adaptation (0.86). Several studies show that the levels of proline are more specifically regulated than the other solutes during adjustment to stress. In earlier studies, it was shown that osmotic adjustment (OA) by tissues of whole plants was almost completely accounted for by the solutes measured. For example, in wheat 60-100% of the decrease in solute potential ($Ø_s$) in response to decreased leaf $Ø_w$ was accounted for by the accumulation of free amino acids and sugars. Similarly, in fully expanded leaves of sorghum, 84 to 100% of the osmotic adjustment (0.7-1.1 MPa) could be accounted for by increases in solutes. The results indicated greater accumulation of solutes in stressed plants which had lower leaf water potential as compared to that in control. In fact, OA increased with the increase in soil moisture stress, because it was a stress induced plant adaptation through accumulation of solutes (Morgan 1984, Ludlow and Muchow 1988).

Leaf gas exchange parameters: Net Photosynthesis (P_n) of leaves of control and water stressed plants (Fig. 4-A) was similar in the control and in stressed plants during the initial stages of stress development up to 93 DAS. P_n rate fluctuated in a narrow range in the control plants during the entire period of study and a slight decrease was observed during the later growth stages. On the contrary, P_n was greatly affected in both mild and severe water stressed plants. A steady decline in P_n was recorded in the mild stress which showed a gradual reduction in P_n from 25.42 to 19.44 ì mol CO_2 m^{-2} s^{-1} at 152 DAS. The reduction in P_n was very steep afterwards and reached 12.42 ì mol CO_2 m^{-2} s^{-1} at 169 DAS (52% reduction). In the severe stress, P_n was

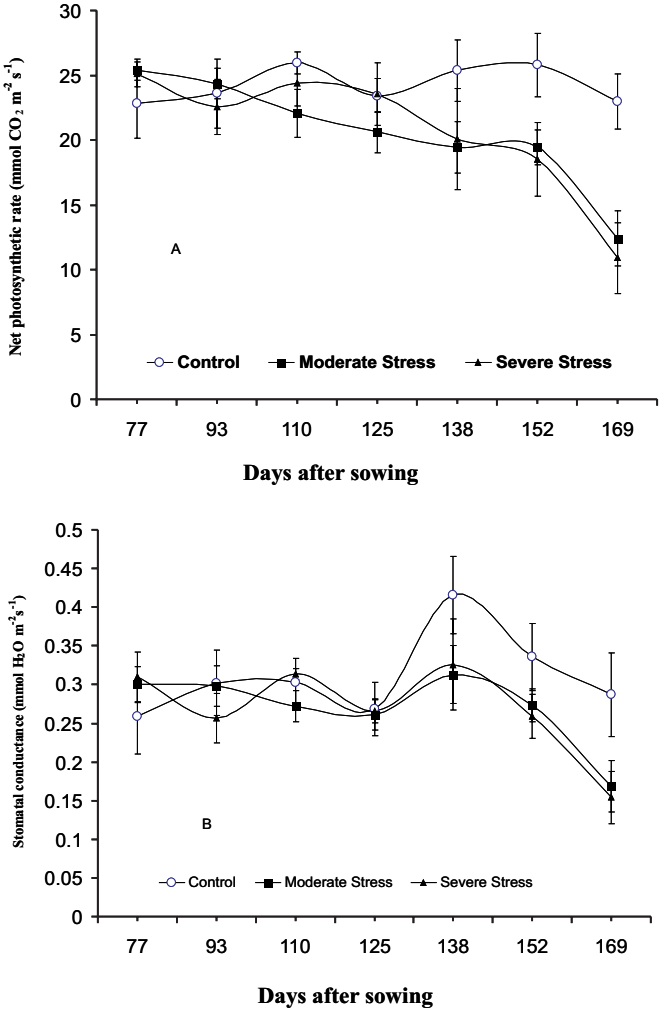

Fig. 4. Changes in net photosynthetic rate (A) and stomatal conductance (B) of *W. somnifera* plants grown under different irrigation regimes. The error bars represent SD (P=0.05).

maintained during the initial stages upto 125 DAS (23.53 ì mol CO_2 m^{-2} s^{-1}) and started declining rapidly as the intensity of stress progressed and reached 10.89 mmol m^{-2} s^{-1} (53% reduction). The impact of soil moisture deficit on carbon assimilation was found be severe even under mild stress due to the damage it causes to the photosynthetic machinery and the photo-inhibition due to decreasing leaf turgor. The reduction in P_n of 50% shows that stress severely affected the process of carbon assimilation which the major source of biomass production.

Leaf conductance (g_s) measured during the course of treatment period (Fig 4-B). The g_s in the control and initial stages of stressed plants remained fairly constant with the values close to 0.3 mol H_2O m^{-2} s^{-1}. It declined to 0.169 and 0.154 H_2O m^{-2} s^{-1} at 169 DAS in moderate and severe stress. The response of plants with reduction in g_s suggests water conservation from partial stomatal closure and loss of water through stomata (Jones *et al*. 1985). Stomatal closure and cuticular resistance prevent excess water loss from the plant. Black *et al*. (1985) recorded lower leaf water potential, turgor potential and stomatal conductance when moisture stress was imposed in groundnut plants, however, stomatal conductance (g_s) was more strongly affected than leaf water status. Stomatal conductance was correlated with leaf water potential and soil water potential. Stomata, then, play a critical role in regulating water flow and maintaining Ø in the plants at levels such that physiological processes are not damaged. Water conservation is a plausible explanation, since measurements of the conductance of leaves were having 58.8% and 53.7% of conductance in non stressed and in the mild and severe stress treatments, respectively.

Chlorophyll Fluorescence Parameters: Leaf chl-a fluorescence parameters were studied at the end of the stress treatment provided in the Table 1. The data revealed that significant changes occurred to Chl a fluorescence in the stressed leaves compared to control.

Table 1. Effect of soil moisture deficit on Chl-a fluorescence kinetics of *Withania somnifera*.

Treatments	Fo	Fm	Fv/Fm	Fv'/Fm'	PhiCO2	qP	NPQ	ETR	PhiPS2
Control	310.7	1872[a]	0.834[b]	0.544[a]	0.022[b]	0.614[a]	1.189	204.0[b]	0.336[a]
Moderate stress	336.9[a]	1902[a]	0.823[a]	0.536[a]	0.019[a]	0.428	1.338[a]	150.4[a]	0.253
Severe stress	324.8	1763	0.816	0.500	0.014	0.400	1.376[a]	124.6	0.209
C.D. (p=0.05)	22.4	78.9	0.010	0.017	0.003	0.180	0.153	19.7	0.083

Dark adopted basal fluorescence (*Fo*) was lowest under control plants. *Fo* increased to the tune of 8% moderate stress compared to control. *Fm* (maximal fluorescence) was higher under moderate stress, however, it was at par with control. A reduction of 6% was observed for *Fm* under severe stress compared to control. *Fv/Fm (*Maximum quantum efficiency of PSII photo chemistry) was altered marginally under stress. There was a reduction of only 1.3% and 2.2% in Fv/Fm under moderate and severe stress compared to control. Whereas, *Fv'/Fm*' (PSII maximum efficiency) which is the PSII operating efficiency if all the PSII centers were 'open' (QA oxidized) was altered to a higher extent under stress. The reduction was to the tune of 1.4% and 8.1% of control in moderate and severe stress, respectively. There was a drastic reduction $PhiCO_2$ and ETR (electron transport rate) in stressed plants. Highest $PhiCO_2$ was recorded in control (0.022), whereas stress plants maintained only 86.4% and 63.3% of $PhiCO_2$ at the end of the treatment period. Similarly ETR got reduced under stress to the tune of 26.3% and 38.9% of control. There was a sharp decline of PhiPS2 in stressed plants. The reduction was 24.7% and 37.8% in moderate and severe stress. The qP (photochemical quenching) was significantly reduced in both stress levels. The reduction was 30.2% and 34.8% in moderate and severe stress compared to control. Concurrently, NPQ (non photochemical quenching) marginally increased from 1.189 in control to 1.338 and 1.376 under moderate and severe stress.

Progressive soil moisture stress did not result in discernible changes in dark adapted fluorescence parameters indicating that water stress had not influenced the primary photochemistry of PS2 and energy distribution within the light-harvesting complex (Flagella *et al.* 1994, Lu and Zhang 1999). However, operating efficiency of photosystem photochemistry was reduced significantly. Loreto *et al*. (1995) observed a decrease in qP under water stress in *Sorghum bicolor* leaves and the results of this experiment was also in line with them. The thermal dissipation was higher under moderate stress and lowest in the severe stress. The reduction in chlorophyll content might be responsible for reduced absorption of light and reduced thermal load in the stressed leaves.

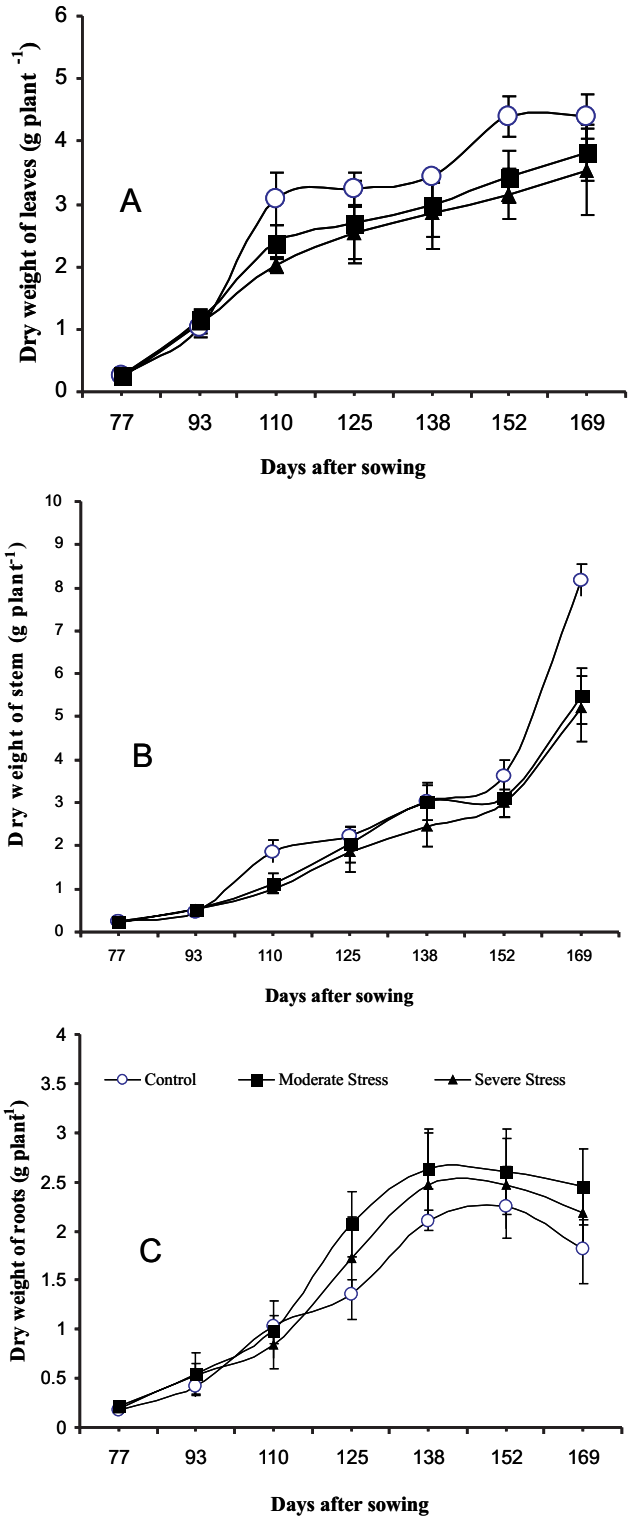

Fig. 4. Changes in net photosynthetic rate (A) and stomatal conductance (B) of *W. somnifera* plants grown under different irrigation regimes. The error bars represent SD (P=0.05).

Biomass accumulation in leaf, stem and root: Dry weight of leaf (DWL) showed an increasing trend throughout the treatment period with double sigmoid growth pattern in control plants (Fig. 4-A). However, stressed plants showed reduced DWL compared to control plants in all stages and DWL steadily increased in plants under mild and severe stress also probably due to production of smaller leaves during the progressive stress period. Dry weight of stem (DWS) did not differ among the plants of control and stress treatment until 152 DAS (Fig. 4-B). It increased steadily in the plants until 152 DAS and increased substantially in the control plants reaching 4.11 g plant^{-1} dry weight of root (DWS). Highest DWR was observed under mild water stress. DWR were 2.10, 2.62 and 2.47 g plant^{-1} under control, mild and severe water stress conditions at 152 DAS, respectively (Fig. 5-C). There was a decrease in DWR in all the treatments afterwards. However, the decrease was more prominent in the control and the least under mild stress.

At late growth stage, the dry matter accumulation in stressed leaves marginally reduced. At 169 DAS, leaf dry weight differed only 20% and 13% in severe and mild stress, respectively compared to control plants. The DWL gain in the stress treatment shows the tolerance of *W. somnifera* to the soil moisture stress by way of adaptation. Since leaves are the primary source for the dry matter accumulation for the plant, the production of new leaves continued in stress, however, the leaves were smaller compared to control. The pattern of stem biomass accumulation was different from the leaves. The gain in fresh and dry weight of stems continued in all the treatments until late growth stages. As the stem weight gain was substantially higher in late vegetative phase, the loss of dry matter accumulation was prominent in the late vegetative phase in stress treatments due to the reduced soil moisture availability. Even at 152 DAS, the severe stress had 82.65% of stem biomass compared to control plants. At 169 DAS, when the intensity was more prominent in severe stress, the gain in stem biomass was restricted and it showed nearly 36% less stem biomass than the control, While, the mild stress had a 33% reduction in stem biomass. The results showed that the stem growth was severely affected than the leaf growth under stress. On the contrary, root length was not significantly affected by the stress treatment. In the stress treatment, preferential accumulation of dry matter occurred in the roots compared to shoots which helps the plant to obtain water as it was increasingly become the limiting resource. The root-shoot ratio was significantly higher at 110 DAS compared to non stress control. Several studies in other crops showed the increased rooting, root length, more number of fine roots at the onset of drought with increased partitioning of biomass accumulation in roots. The increased root growth and root system development helps the plant to explore the wider area of soil into the deeper soil layers for water. In our study, the response of plant to drought signal was initiated at the early stage itself and DWR showed significant increase over control at 138 DAS. Allen *et al*. (1976) concluded from measured soil water extraction that during water stress, roots of groundnut in lower depths continue to grow deeper even though vegetative growth appears to stop.

In *W. Somnifera*, the dry matter production, physiological functions and chlorophyll fluorescence parameters were affected adversely by the soil moisture deficit, however the root dry matter yield was higher in plants under moderate stress compared to no stress control due to the increased biomass partitioning in roots. The root quality in the well watered control was poor due to the branching of the roots and higher number of fibrous roots. The market acceptability for fibrous roots are less and hence moderate stress produce the good quality roots even though the plant total biomass is lesser compared to no stress control. It is concluded from the results that increased root biomass partitioning and higher root dry weight under moderate soil moisture stress helped in off-setting the deleterious effect of water stress with increased root quality in *W. somnifera*.

ACKNOWLEDGEMENT

Authors are thankful to the Director, DMAPR for providing facilities for this work.

REFERENCES

Alberte, R.S. and Thornber, J.P. (1977). Water stress effects on the content and organization of chlorophyll in mesophyll and bundle sheath chloroplasts of maize. *Plant Physiol*. **59:** 351-353.

Allen, L.H., Boote, K.J. and Hammond, L.C. (1976). Peanut stomatal diffusive resistance affected by soil water and solar radiation. *Proc. Soil Crop Sci. Soc. Fla.* **35:** 42-46.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts and polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* **24:** 1-15.

Bates, L.S., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline in water stress studies. *Plant Soil*. **39:** 205-208.

Black, C.R., Tang, D.Y., Ong, C.K., Solon, A. and Simmonds, L.P. (1985). Effects of soil moisture on water relations and water use of groundnut stands. *New Phytol.* **100:** 313-328.

Fischer, R.A. and Wood, J.T. (1979). Drought resistance in spring wheat cultivars. III. Yield association with morpho-physiological traits. *Aust. J. Agri. Res.* **30:** 1001-1020.

Flagella, Z., Pastore D., Campanile, R.G. and Fonzo, N.D. (1994). Photochemical quenching of chlorophyll fluorescence and drought tolerance in different durum wheat (*Triticum durum*) cultivars. *J. Agri. Sci.* **122:** 183-192.

Handa, S., Bressan, R.A., Handa, A.K., Carpita, N.C. and Hasegawa, P.M. (1983). Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. *Plant Physiol.* **73:** 834-843.

Jones, P., Jones, J.W. and Allen, L.H. (1985). Seasonal carbon and water balances of soybeans grown under stress treatments in sunlitchambers. *Trans. ASAE.* **28:** 2021-2028.

Loreto, F., Tricoli, D. and Marco, D.G. (1995). On the relationship between electron transport rate and photosynthesis in leaves of the C4 plant *Sorghum bicolor* exposed to water stress, temperature changes and carbon metabolism inhibition. *Aust. J. Plant Physiol.* **22:** 885-892.

Lu, C. and Zhang, J. (1999). Effects of water stress on photosystem II photochemistry and its thermostability in wheat plants. *J. Exp. Bot.* **50:** 1199-1206.

Ludlow, M.M. and Muchow, R.C. (1990). A critical evaluation of traits for improving crop yields in water-limited environments. *Adv. Agron.* **43:** 107-152.

Morgan, J.M. (1984). Osmoregulation and water stress in higher plants. *Annu. Rev. Plant Physiol.* **35:** 299-319.

Ryan, J. (2002). Available Soil Nutrients and Fertilizer use in Relation to Crop Production in the Mediterranean Area. In "*Soil Fertility and Crop Production*". (eds.), Krishna, K. R. Science Publ., Enfield, New Hampshire, USA. pp. 123-246.

van Kooten, O and Snell, J.H.F. (1990). The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosyn. Res*. **25:** 147-150.

Wacker, B. (1999). Generating soil moisture characteristics. *Soils News* **3:** 2.

Yamaguchi-Shinozaki, K. and Shinozaki, K. (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Ann. Rev. Plant Biol*. **57:** 781-803.