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Effect of plant population and soil moisture stress on herbage yield and andrographolide content in *Andrographis paniculata*

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ABSTRACT

Spacing and irrigation levels influenced dry weight of leaves and fresh and dry weight of stems significantly. Plant spacing of 15 x 15 cm produced higher herbage yields of 707.8, 742.3 and 690.6 g.m^{-2} in well-watered control, mild and severe water stress treatments, respectively. Low yields were recorded under 30 × 45 cm spacing in control, mild and severe water stress treatments, respectively. Among the treatments, andrographolide content in the leaves was highest in severely water stressed plants. Increasing the plant spacing positively influenced the andrographolide content in the leaves under the severe water stressed plants with highest andrographolide content in wider spacing (30 × 45 cm). In the stems, highest andrographolide content of 1.75% was in 15 × 15cm plant spacing and lowest was in 30 × 45cm under no stress control. Similar trend was noticed in mild and severe water stressed plants under various spacing treatments. Andrographolide yield from herbage was maximum in control followed by mild and severe water stressed conditions in the closer spacing. Lowest yield of andrographolide was recorded under 30 x 45 cm spacing under control condition. Dry matter yield was lower under mild and severe water stress conditions even under closer spacing, but the loss of andrographolide yield was partly compensated by its higher content. In *A. paniculata*, increased accumulation of andrographolide in leaves of mild and severe water stressed plants compared to well watered control may be attributed to the response of stress signalling.

Key words: *Andrographis paniculata,* plant spacing, moisture stress, herbage yield, andrographolide content.

INTRODUCTION

Andrographis paniculata Nees. (Sanskrit Name: Kalmegh) is an important medicinal herb used extensively as a hepatoprotective drug, anti-inflammatory agent and against stomach ulcers in India and many south-eastern countries. It is cultivated in many parts of India under rainfed condition with supplemented irrigation. Due to uneven rainfall and shortage of irrigation water, the crop experiences intermittent periods of water stress. Soil water deficit reduces the dry matter yield of many crops primarily by reducing the whole canopy absorption of incident PAR (photosynthetically active radiation) either by limitation of leaf area expansion or by leaf wilting or by early leaf senescence (Jones *et al.,* 6 ; Wolfe *et al.,* 15). The efficiency with which absorbed PAR is used by the crop to produce new biomass is also adversely affected by soil water stress. Shibles and Weber (12) found that rate of dry matter accumulation was linearly related to the percent interception of incoming solar radiation. They also observed that a leaf area index (LAI) of approximately 3.2 was required to achieve 95% light interception and 95% of maximum dry matter production in soybean. Light interception of 95 % is important because it is the value at which crop should theoretically achieve canopy coverage, maximum canopy photosynthesis at the developmental stage, and maximum yield for the environmental condition (Westgate, 14).

In addition to other factors, which influence the dry matter yield in field crops, plant population density plays a crucial role in obtaining maximum dry biomass from a unit area. The effect of plant population density on field and horticultural crops has been studied extensively by various workers (Wade *et al*., 13; Lege *et al*., 8; Cuomo *et al.*, 1). In contrast, very little is known about the effect of plant density on herbage yield and active principle content of medicinal plants. For field crops such as soybean, maize and sorghum, grain yield is generally maximised by adjusting population density with the moisture conditions (Sanderson *et al*., 11). High population density stands utilise available moisture and nutrients more quickly than sparsely populated stands (Jones and Kielly, 7). Rationalisation of use of irrigation water is a priority area, as water is becoming a scarce resource in most parts of the country. Increasing the productivity of the crop under water-limited environment has been a thrust area for several decades globally. Several approaches were made for selection of drought tolerant varieties and breeding for transgenic plants for increased osmotic adjustment which in turn protects the plant during water stress. Higher biomass production was achieved in many field crops including wheat and rice through selection and breeding for drought tolerance. It is imperative to understand the crop physiology of any specific crop under limited water

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availability for improvement in drought tolerance. Understanding crop response under challenging environment provide valuable insight for selection of appropriate genotypes and breeding for drought tolerance in terms of biomass production, partitioning, changes in biochemical constituents, etc. Currently, the information on the effect of soil water deficit on *A. paniculata* herbage yield and andrographolide content is lacking. The objective of this study was to determine the effects of soil water deficit and plant density on herbage yield and andrographolide content in *A. paniculata*.

MATERIALS AND METHODS

The field experiments were carried out at the experimental farm of National Research Centre for Medicinal and Aromatic Plants (NRCMAP), Anand, Gujarat, India (latitude 07° 15'N, longitude 78° 14'E.) in two consecutive years (2002 & 2003). The soil was sandy loam with neutral pH (pH 6.5 - 7.5) low in organic carbon (0.5%). The seeds of a local cultivar were sown in nursery in the month of May in both the years. One and half month old seedlings were transplanted in the field on 5th July 2002 and 8th July 2003 in respective years in a split-plot design with irrigation as main plot and spacing as subplot. In 2002, there were two irrigation levels based on the soil moisture content in order to generate no stress and severe stress condition with four plant spacing treatments (15cm × 15cm, 15cm × 30cm, 30cm × 30cm and 30cm × 45cm). In 2003, three irrigation levels were given to create differential soil moisture regimes with same four plant spacing as in the previous year. The crop received 416.0mm and 914.5mm rainfall during 2002 and 2003 respectively.

Measured amount of air dried soil was taken in air tight container and it was added with de-ionized water to get appropriate soil moisture content. The samples were kept overnight to reach equilibrium. The water potential of the soil sample was measured by using a soil chamber psychrometer (Wescor Inc, USA). The water potential was measured for a range of soil moisture content. The resultant water potential data were plotted against the each soil moisture content. The standard curve was used to calculate the soil moisture potential of soil samples collected from the plots.

Canopy temperatures (℃) were measured using model AG-42D (Fullerton, USA) portable hand-held infrared thermometer. The instrument has field view of 15℃, a sensing window of 10.5 to 12.5 mm and a resolution of 0.1℃. In each measurement the infrared thermometer was held above the plant canopy at an angle of 15℃ below the horizontal so that plant parts, but no soil was viewed. In each measurement, three canopy temperature measurements were taken from the east and three reading from the west and then averaged. Andrographolide content in leaf and stem was estimated for the second year crop. The sample sizes of 0.5 g dry powder from both leaves and stems of three month old plants were extracted exhaustively with a 1:1 mixture of dichloromethane and methanol by cold maceration (Rajani *et al*., 10). The extract was filtered and the solvent removed on rotary evaporator. The dark green crystalline residue obtained was washed with toluene. After complete removal of toluene, the residue was dissolved in 80% methanol. This solution was filtered through 0.45 ìm syringe filter and thereafter 10 µl was used for analysis by HPLC (Shimadzu, Japan) having LC-10AD pumps and SPD-10A UV-VIS Detector. The LiChrospher RP-18e column was used for analysis at 30-35℃ ambient temperature. Standard andrographolide was used (M/S Sigma-Aldrich, USA) for identification and quantification of andrographolide extract.

RESULT AND DISSCUSSIONS

Soil water potential was maintained within –1.0 to –1.5 MPa during the growing period in well watered control plots (Fig. 1). In the water stress treatment plots the soil water potentials were between –2.5 MPa to –3.0 MPa at a soil depth of 15cm during the measurement period in the year 2002. The soil moisture depletion resulted in mild to severe drought stress to plants in 2002. In 2003, similar trend was observed in control treatment. Mild to severe water stress was evident in the plants under water stress treatments as the soil water potential dropped to –2.0 to –2.2 MPa and –2.5 MPa to –3.0 MPa in irrigation level 2 and level 3, respectively.

The atmosphere - canopy temperature difference was in the range of 5.0 - 5.5℃ at 62 DAP (days after planting) in all the treatments. There was no significant difference for the canopy temperature depression among the treatments in the early growth stages. At later growth stages, the canopy temperature was maintained cooler in control plants within the range of 3 - 5℃ at 90 and 120 DAP (Fig. 2). The canopy temperature was higher than the prevailing ambient temperatures at noon in the irrigation schedule 2 and 3. As the leaf area increased in the later growth stages, the transpiration demand tend to be higher in the mild and severe drought stressed plants. The higher than normal canopy temperature in irrigation schedules 2 and 3 indicated the quantum of stress experienced by the plants. The restricted water availability in the root zone negatively influenced the evapo-transpirational cooling in the canopy thereby increasing the canopy temperature. A linear relationship between canopy minus air (Tc-Ta) temperature differences and vapour pressure deficit (VPD) of the air for well irrigated plants transpiring at potential rate during the day light hours was observed by Idso et al. (5).

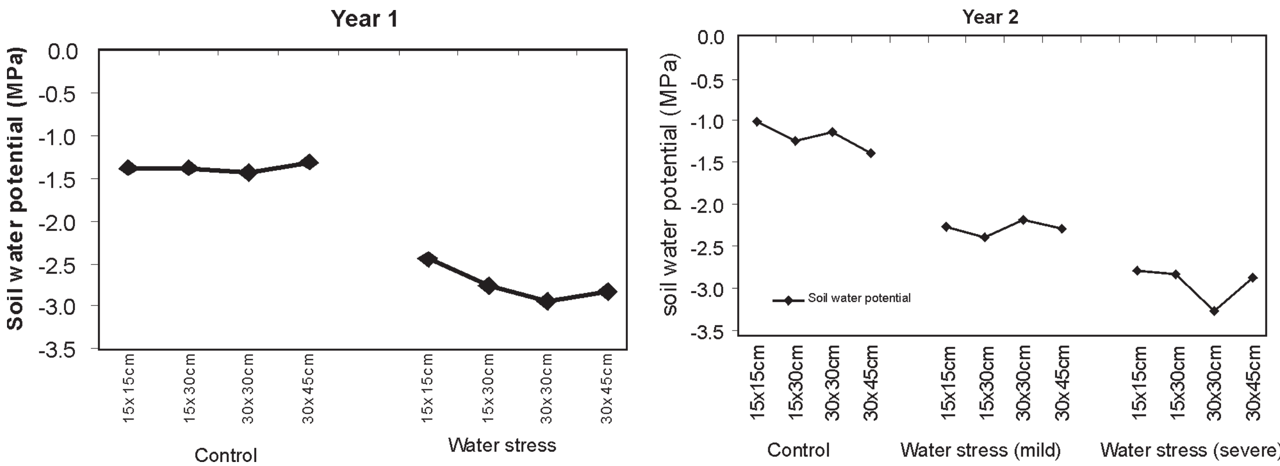

Fig. 1. Soil water potential changes in control water stress conditions.

Heermann and Duke (4) used the average Tc-Ta values between treatment plots and adjoining well-watered areas to study crop water stress under limited irrigation for corn plants. They reported that the average temperature difference (Tc-Ta) elevation was linearly related to irrigation and to relative dry matter yield. They concluded that average Tc-Ta> 1.5℃ was significantly correlated with grain yield reduction.

The soil water potential significantly influenced dry weight of leaves, stem and whole plants in the year

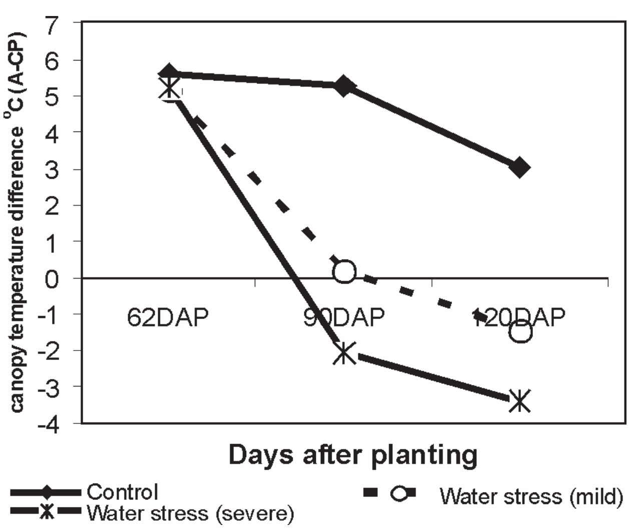

Fig. 2. Effect of water stress on canopy temperature difference in *A. paniculata* during growing period.

2002 (Table 1). Plant spacing of 15 × 15 cm was found to be superior in terms of all the parameters studied. Significant interaction effect was found in the leaf area and dry weight of leaves for levels of irrigation and spacing. Highest fresh leaf weight was obtained in closer plant spacing of 15 × 15 cm in both control (229.1 g.m^{-2}) and water stressed (189.2 g.m^{-2}) treatments in 2002. Spacing and irrigation levels influenced dry weight of leaves and fresh and dry weight of stems significantly. Maximum dry weight of leaves (113.5 g.m^{-2}), fresh weight of stem (1080.1 g.m^{-2}), dry weight of stem (620.5 g.m^{-2}) and dry weight of whole plant (734.1g.m^{-2}) was obtained in control treatment with 15 × 15 cm plant spacing.

In the year 2003, plant height, fresh weight and dry weight of leaves significantly varied among the irrigation levels and plant population density. Highest fresh and dry weight of leaf was recorded under 15 × 15cm spacing in control (Table 2). Dry herbage yield was not significantly influenced by irrigation schedule alone. Plant spacing of 15 × 15 cm produced higher herbage yields of 707.8, 742.3 and 690.6 g.m^{-2} in well-watered control, mild and severe water stress treatments respectively. Lowest yield of 211.2, 198.7 and 198.4 g.m^{-2} were recorded under 30 × 45 cm spacing in control, mild and severe water stress treatments respectively. Fresh and dry weight of stem along with whole plant herbage yield was not significantly influenced by irrigation levels. However, plant population density influenced the herbage yield in both the years. The interaction of irrigation and spacing was found not significant for the stem and whole plant herbage yield. Maximum herbage yield was obtained in the 15 cm × 15 cm spacing in all soil moisture levels (Table 2). The soil moisture level did not influence most of the parameters in 2003. It might be due to the high rainfall received during this year (914.5 mm) compared to the previous year (416.0 mm). The rainfall received during the early and mid growth periods resulted in the better growth and contributed to the final dry matter yield.

The dry matter contribution from the leaves to the final herbage yield under drought is partly determined

Table 1. Plant characteristics of *Andrographis paniculata* under different irrigation levels and plant population density.

Treatment	Spacing (cm × cm)	Fresh weight of leaves (gm^{-2})	Dry weight of leaves (gm^{-2})	Fresh weight of stem (gm^{-2})	Dry weight of stem (gm^{-2})	Fresh weight of whole plant (gm^{-2})	Dry weight of whole plant (gm^{-2})
Control	15×15	229.1	113.5	1080.1	620.5	1309.1	734.1
	15×30	135.5	56.2	766.5	367.3	902.0	423.5
	30×30	80.4	32.3	591.8	321.5	672.2	353.8
	30×45	43.8	23.1	613.0	290.0	656.9	313.1
Water stressed	15×15	189.2	77.9	989.0	478.3	1178.2	556.2
	15×30	125.8	43.1	548.5	255.3	674.3	298.4
	30×30	49.2	21.3	348.9	161.9	398.1	183.1
	30×45	41.5	17.5	346.0	152.2	387.5	169.8
CD (a) at 5%	49.4	15.7	286.6	41.6	ns	35.8	
CD (b) at 5%	33.2	9.14	133.8	32.5	144.3	35.9	
CD (axb) at 5%	6.24	1.14	51.02	23.16	ns	4.48	

Table 2. Plant characteristics of *Andrographis paniculata* under different irrigation levels and plant population density.

Irrigation	Spacing (cm×cm)	Plant height (cm)	Fresh weight of leaves gm^{-2}	Dry weight of leaves gm^{-2}	Fresh weight of stem gm^{-2}	Dry weight of stem gm^{-2}	Fresh weight of whole plant gm^{-2}	Dry weight of whole plant gm^{-2}
Control	15×15	55.5	547.58	213.69	972.91	597.52	1501.35	707.89
	15×30	54.8	354.05	139.59	618.75	321.16	1003.64	416.75
	30×30	54.1	184.53	62.66	464.33	193.03	642.64	248.36
	30×45	56.5	158.92	67.09	319.88	173.70	498.09	211.20
Water stress (mild)	15×15	55.5	332.86	127.01	1276.44	615.34	1604.97	742.35
	15×30	58.4	243.50	93.50	678.26	291.79	885.28	399.96
	30×30	54.1	145.59	47.26	376.44	204.25	502.30	255.18
	30×45	56.8	122.04	38.09	348.96	173.01	464.49	198.76
Water stress (severe)	15×15	46.0	221.39	84.26	1158.45	518.39	1298.59	690.65
	15×30	45.3	195.76	64.86	560.89	339.72	730.77	331.25
	30×30	47.7	126.06	44.04	476.98	239.49	608.04	301.86
	30×45	49.1	118.51	46.18	278.98	157.18	389.06	198.42
CD (a) at 5%		5.75	58.81	27.39	NS	NS	NS	NS
CD (b) at 5%		NS	34.08	15.75	126.97	47.18	130.58	85.44
CD (axb) at 5%		NS	5.22	2.41	NS	NS	NS	NS

by the drought induced leaf senescence and abscission. Drought-induced leaf senescence contributes to nutrient remobilisation during stress, thus allowing the rest of the plant (*i.e.,* the youngest leaves, fruits or flowers) to benefit from the nutrients accumulated during the life span of the leaf. In addition, drought-induced leaf senescence, especially when accompanied by leaf abscission, avoids large losses through transpiration, thus contributing to the maintenance of a favourable water balance of the whole plant. (Munné-Bosch and Alegre, 9). The canopy coverage in wider spacing (30 × 30 cm) and (30 × 45cm) was sparse and the plants did not produce enough source leaves to utilise the available light for dry matter production in these treatments. Even under well-watered control treatment the dry matter yield was markedly reduced by wider plant spacing and plants did not compete for the available light. *A. paniculata* is found mostly as a under-story in wild and the genotypes used for cultivation are not selected for saturating light conditions in the field.

The increase in herbage yield is attributed to the closer plant spacing resulted in maximum canopy coverage which increased the per unit biomass production. In the wider spacing treatments 30 × 30 cm and 30 × 45 cm, the light interception by the plants did not reach maximum even at the later stages. The decrease in dry matter yield per plant in mild and severe water stress was more than off set by plant population.

Andrographolide content was significantly higher under water stressed plants compared to control plants at all plant population densities. Maximum andrographolide content (2.85 – 3.30%) in the leaves was observed in severely stressed plants. Increasing the plant spacing positively influenced the andrographolide content in the leaves under the severe water stressed plants with highest andrographolide content (3.30%) in wider spacing (30 × 45 cm) and lowest (2.85%) in closer spacing (15 × 15cm). In the mild stress and control, leaf andrographolide content was lower under wider spacing compared to narrow plant spacing. Leaf andrographolide content was significantly lower in 30 × 45 cm spacing in both control and mild water stressed plants which were 1.15% and 1.83% respectively. Under mild water stress, significantly higher andrographolide (2.74%) was recorded in 15 x 30cm spacing. However, under control condition, 30 × 30 cm gave the higher leaf andrographolide content.

Significant changes in andrographolide content were recorded for the stems under different plant spacing. A decreasing trend was noted for andrographolide content towards the wider spacing in control and water stressed plants. In the control, highest andrographolide content of 1.75% was in 15 × 15cm plant spacing and lowest was in 30 × 45cm (0.59%). Similar trend was noticed in mild and severe water stressed plants under various spacing treatments. Highest content of 1.36% and 1.27% were recoded in 15 × 15cm of mild and severely stressed plants respectively.

Andrographolide yield from herbage was highest in the closer spacing (15 × 15 cm) in control and water stressed plants among the different plant spacing studied. In the closest spacing, the yield was maximum in control (14.45 g m^{-2}) followed by mild (11.40 g m^{-2}) and severe (9.05 g m^{-2}) water stressed condition. Lowest yield of andrographolide was recorded under 30 × 45 cm spacing under control condition. Under same spacing level, higher yields of 1.91 g m^{-2} and 2.73 g m^{-2} in mild and severe stress conditions.

Water stress resulted in increased andrographolide content in leaves. Severe water stress caused higher accumulation in the leaves. On the contrary, the content did not increase in the stems and decreased with increase in the stress level. The yield of andrographolide

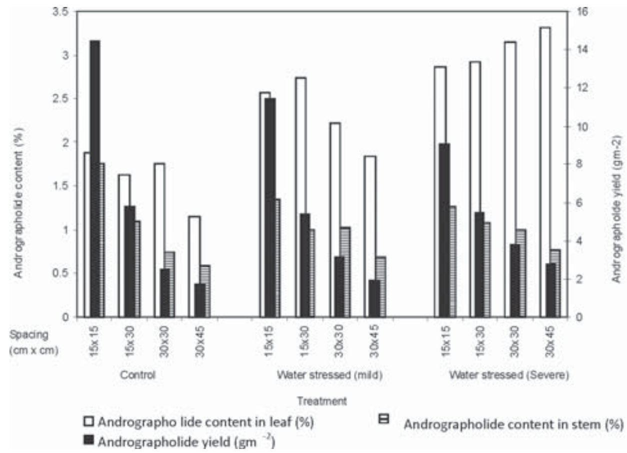

Fig. 3. Andrographolide content in leaf, stem and andrographolide yield from herbage of *Andrographis paniculata* under control and water stressed condition.

from total herbage was highest under the no stress control because of the higher herbage yield. Even though the dry matter yield was lower under mild and severe water stress conditions, the loss of andrographolide yield was partly compensated by its higher content. Environmental stresses have profound effect on level of many secondary metabolites in numerous plants (Gershenzon, 3). The effect of water stress on the synthesis and accumulation defencive compounds depends on the roles they play in the system, localisation and pattern of sequestration of compounds. In some conifer species, the induced response to bark beetle attack is diminished under stress condition. A threshold of -2 MPa xylem water potential was found to cause susceptibility to fir engraver attacks in water stressed white fir stands. This water stress induced susceptibility was accompanied by diminished mono-terpene oleoresin content in attack sites (Ferrel, 2). A common signal for various environmental stresses is abscissic acid (ABA) which regulates the expression of several hundred genes related to the various metabolic pathways. Since these genes are activated or over expressed in water stress, the production and accumulation of vast array of secondary metabolites occur in various plant species. In *A. paniculata*, increased accumulation of andrographolide in leaves of mild and severe water stressed plants compared to well watered control may be attributed to the response of stress signalling

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REFERENCES

1. Cuomo, G.J., Redfearn, D.D. and Blouin D.C. 1998. Plant density effect on tropical corn forage mass, morphology, and nutritive value. *Agro. J.* **90**: 93-96.
2. Ferrel, G.T. 1978. Moisture stress threshold of susceptibility to fir engraver beetles in pole-size white fir. *Forest Sci.* **24**: 85-92.
3. Gershenson, J. 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. **In:** *Phytochemical Adaptations to Stress* (Eds., B.N. Timermann, C. Steelink, F.A. Loewus), Pienum Press, New York, pp: 273-320.
4. Heermann, D.F. and Duke, H.R. 1978. Evaluation of crop water stress under limited irrigation. ASAE, St. Joseph, MI. ASAE paper 78-2556.
5. Idso, S.B., Jackson, R.D., Pinter, P.J. Jr., Reginato, R.J. and Hatfield, J.L. 1981. Normalising the stress-degree-day parameter for environmental variability. *Agri. Meteorol.* **24**: 45-55.
6. Jones, J.W., Zyr, B. and Bennett, J.M. 1986. Interactive effects of water and nitrogen stresses on carbon and water vapour exchange of corn canopies. *Agri and Forest. Meteorol*. **38**: 113-26.
7. Jones, O.R. and Kielly. 1998. Row width and plant density effect on texas high plains sorghum. *J. Prod. Agri.* **4**: 613-21.
8. Lege, K.E., Smith, C.W. and Cothren, J.T. 1993. Planting date and planting density effects on condensed tannin concentration of cotton. *Crop Sci*. **33**: 320-24.
9. Munné-Bosch, S. and Alegre, L. 2004. Die and let live: Leaf senescence contributes to plant survival under drought stress. *Funct. Plant Biol.* **31**: 203-16.
10. Rajani, M., Neeta, N. and Ravishankara, M.N. 2000. A rapid method for isolation of andrographolide from *Andrographis paniculata* Nees (Kalmegh). *Pharm. Biol.* **39**: 204-09.
11. Sanderson, M.A., Jones, R.M., Read, J.C. 1996. Management of forage sorghum: Nitrogen, plant density and irrigation effects on yield and quality. *Texas J. Agri. Resources*. **9**: 61-78.
12. Shibles, R.M. and Weber, C.R. 1996. Interception of solar radiation and dry matter production by various soybean planting patterns. *Crop Sci.* **6**: 55-59.
13. Wade, L.J., Norris, C.P., Walsh, P.A. 1988. Effect of suboptimal plant density and non-uniformity in plant spacing on grain yield of raingrown sunflower. *Aust. J. Exp. Agri.*. **28**: 617-22.
14. Westgate, M.E. 1999. Managing soyabeans for photosynthetic efficiency. **In**: *World Soyabean Reserach Conference- 4th* (Ed., Kauffman), Chicago-IL. Superior Print. Champaign. pp 223-28.
15. Wolfe, D.W., Henderson, D.W., Hsiao, T.C. and Alvino, A. 1988. Interactive water and nitrogen effect on senescence of maize: II. Photosynthetic decline and longevity of individual leaves. *Agronomy J.* **80**: 865-70.

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