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The Effects of Water Deficit Stress, Cold Shock and Salicylic Acid on Maize Hybrids Growth Parameters

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ABSTRACT: In order to evaluate the effects of cold and water deficit stress as well as salicylic acid foliar application on growth parameters of maize hybrids an experiment was conducted at the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran in 2014 and 2015. The results revealed that in both years there was no difference between cold stressed and none stressed plants in terms of growth parameters. In all hybrids, the crop growth rate and dry matter accumulation significantly decreased through reducing leaf expansion caused by severe water deficit stress (50% of water requirement). The maximum leaf area index (3.48) was found in single cross 704, grown under full irrigation condition. Stress induction at seedling and fast growing stages, increased crop growth rate and relative growth rate at the end of growing period. Salicylic acid application increased leaf area and dry matter production and could improve growth parameters under stress conditions.

Keywords: Maize, Crop growth rate, Net assimilation rate, Leaf area index, Dry matter

INTRODUCTION

Maize (*Zea mays* L.) commonly known as corn, is a grain plant belonging to the Poaceae family. Maize has a relatively short growth period; however, it is recognized as a high yield crop. Maize is also known as the second crop in the word in terms of production per cultivation area after wheat and the third crop in the world in terms of cultivation area after wheat and rice (FAO, 2014). Maize cultivation area in Iran is increasing over the years. In 2014, grain maize and forage maize covered an area of thousand hectares and 203 thousand hectares, respectively and produced 1.7 million ton grain and 9.8 million ton forage. According to the statistics (2014), maize cultivation area and production in Razavi Khorasan Province, located in Northeast of Iran, were reported as 16.5,000 hectare and 762.7 ton, respectively. Maize is native to tropical regions so it is a cold sensitive plant. Nevertheless, early seed sowing in spring conserves the crop against pests and diseases as well as heat waves during flowering and pollination (Tollenar and Wu, 1999). However there is a risk of cold stress in spring. It has been reported that cold stress causes several physiological impairments in cold sensitive species and reduce growth rate (Lukatkin *et al.,* 2012). For instance, cold stress in maize reduces photosynthesis and leaf growth (Nie *et al.,* 1992). In arid and semiarid regions of the world, water deficit stress, as the main restricting factor in agriculture, prompts scientists to pay more attention to the issue (Yadav and Bathagar, 2001). In a study, it has been mentioned that maize can tolerate water shortage at early stages of its growth without yield loss, but water deficit stress at the end of vegetative growth or during reproductive may considerably reduce final yield (Mirhadi, 2001). On the other hand, there is a report stating that water deficit stress reduces maize growth and development, dry matter allocation and final seed yield irrespective of the growth stage, during which water stress occurred (Ming Yang and Hsiang, 1992). Generally, yield loss depends on the growth stage in which water stress occurred, water deficit stress duration and finally stress intensity (Ming Yang and Hsiang, 1992). Rezaverdinejad *et al.,* (2006) found that water deficit stress during maize vegetative growth and flowering stage reduced seed yield by 28 and 29 percent compared with full irrigation treatment. The effect of water deficit stress on leaf area index was studied by Ehsanzadeh and Nouriazhar (2007) who studied maize hybrids growth parameters in response to different irrigation regimes. They have also found a positive and significant correlation between leaf area index and dry matter yield in maize hybrids.

In a similar study, Saberali *et al.,* (2007) stated that maize leaf area index reaches its maximum at silking stage (the emergence of silks beyond the tip of the ear husk) and then decreases due to leaf abscission. Different varieties increase their biomass through increasing leaf area and dry matter so there is no significant difference between them. However, there is a significant difference in allocating dry matter to leaf area or leaf dry weight (Gonzalo *et al.,* 2006). It has been reported that leaf number in maize is controlled by genetic makeup and is a constant trait, thus, leaf number increases with increasing plant population density which in turn leads to higher leaf area index (Gonzalo *et al.,* 2006). In a similar study, Mirhadi (2001) have stated that in different varieties, the crop growth rate at the flowering stage is higher than that at early or late growth stages, in other words, at each growth stages, in which assimilation rate is high the crop growth rate is high too. In addition, Sayer (1994) has shown that water deficit stress reduces leaf area index and leaf expansion rate in sorghum. By contrast, increased irrigation has been found to have a significant effect on growth parameters in soybean as reported by Yazdani *et al.,* (2007). Reduced maize leaf area index due to water deficit stress has been previously reported by Nouri Azhar and Ehsanzedeh (2007). Salicylic acid is a phenolic compound manufactured by many plant species. It is known as a plant hormone (Kang, 2003) that boosts the defence system of plants after an attack by a pathogen or exposure by environmental stresses (Raskin, 1992). Salicylic acid plays a critical role in activating plant defence responses. For instance, it is found to have a key role in increasing cold and drought stress (Hayat *et al.,* 2005). It has been reported that exogenous application of salicylic acid increases plant dry weight (Singh and Usha, 2003). Similar results have been found in barley (Metwally *et al.,* 2003) and maize (Khodary, 2004). Understanding how seed yield is controlled by growth parameters is a major challenge in predicting crop yield. Total dry matter (TDM) accumulation, leaf area index (LAI), crop growth rate (CGR), relative growth rate (RGR) and net assimilation rate (NAR) are the most important growth parameters to evaluate crops potential in efficient use of available resources such as water, nutrient and light (Soleymanifard *et al.,* 2011). The current study was aimed to evaluate the role of salicylic acid in mitigating harmful effects of cold and water deficit stress in maize hybrids.

Material and methods

The current experiment was carried out at Faculty of Agriculture, Ferdowsi University of Mashhad, Iran in 2014 and 2015. A randomized complete block design with split-split plot arrangement with three replicates was used to investigate the experimental factors. In the first year, cold stress at two levels (no stress and stress at four-leaf stage), water deficit stress at three levels (full irrigation, 75% of water requirement (mild stress) and 50% of water requirement (severe stress)) and maize hybrids at three levels (single cross 704, single cross 400 and single cross 260) were allocated to main plots, sub plots and sub-sub plots, respectively. In the second year, different concentrations of salicylic acid (0 M, 200 μM and 400 μM) as a sub-sub factor were applied on single cross 400 as a superior hybrid. This hybrid was selected as the highest seed yield and water use efficiency was related to this hybrid. All foliar applications were performed before cold stress inducing and during crop growth period fortnightly. Maize seeds were sown in paper pots filled with a mixture of sand, perlite, field soil and peat moss (1:1:1:1; w: w) at depth of 5 cm in May 2014 and 2015. The pots were placed in the glasshouse and watered properly until seedling establishment. The light intensity was uniform across the glasshouse. The day/night temperature was set to 25/15°C and relative humidity was about 70%. At four-leaf stage seedlings were transferred into the cold room for cold exposure. The room temperature decreased from 25°C to 5°C at an even pace of 2° C per hour. Plants were exposed to the cold stress for 12 h and then were removed from the room and planted in the field. The field was prepared using moldboard plough and disk. The dimension of each plot was 4 m long by 3 m wide consisting of 4 planting row (0.75 m apart). Planting density of 85,000 plants per ha-1 was used for single cross 260 (early mature hybrid) while single cross 400 and single cross 704 were planted at 75,000 plants per ha^{-1}. Prior to filed preparation soil samples were collected from the depth of 0-60 cm to determine physicochemical properties. Weeds were controlled manually during growing season. Irrigation was done equally for all plots during two first weeks. After two weeks irrigation treatments were applied. Irrigation water transport was done using pipeline network systems equipped with water counter. Amount of required water for each treatment was calculated using following equation.

$$WR = (Etc+Ro-Pe-CR) / (Ei/100)$$

Where WR: maize water requirement (mm), Etc: maize evapotranspiration (mm), Ro: runoff (mm), Pe: effective rainfall (mm), CR: capillary rise (mm) and Ei: efficiency of irrigation (%).

Due to the low water table and closed irrigation runoff was considered to be minimal and was not factored into the water-budget estimates.

Reference evapotranspiration (ETo) was calculated based on the Penman-Monteith equation according to the FAO guidelines using weather station data. Maize evapotranspiration was calculated according to the following equation (Aleen *et al.,* 1998).

$$ETo \times Kc = Etc$$

Irrigation efficiency (Ei) was considered as 90% considering due to closed irrigation and using the pipeline. Growth degree day was calculated using the following equation (Rusell *et al.,* 1984).

$$GDD = \Sigma\ [((T_{max} + T_{min})/2) - T_b]$$

Where GDD: growth degree day; T_{max}: maximum day temperature; T_{min} minimum day temperature, Tb: base temperature (10°C for maize) (Sit and Costello., 1994). Total dry weight changes per unit area were fitted with temperature coefficient and then growth parameters were calculated based on the functional method (Yusuf *et al.,* 1999). To gain the best model, natural logarithm of the data was calculated and then least square was used to determine the polynomial mathematical model. The following models showed the best coefficient of determination (r2) to predict LAI, TDW, CGR, RGR and NAR toward GDD.

LAI = exp(ax2 + bx + c)
TDM = exp(ax2 + bx + c)
CGR= d (TDM)/dt= (2ax + b) × (exp (ax2 + bx + c))
RGR = 2ax + b
NAR= LAI / CGR

RESULTS

A. First year

Total dry weight. There was a similar pattern found among maize hybrids grown under different irrigation regimes in terms of dry matter accumulation. For instance, dry matter accumulation showed a cumulative and fast increase from early growth stages (3-4 leafy stage) to complete flowering stage (silking and full pollination) followed by a gradual reduction due to leaf abscission. From the results illustrated in Fig. 1, water shortage could reduce dry matter accumulation in maize hybrids which might be due to lower leaves abscission, a higher rate of respiration, secrete of inhibitor hormones and reduced leaf duration. The highest dry matter was related to single cross 704 grown under full irrigation treatment and no cold stress.

Leaf area index. As shown in Fig. 2, in all treatments leaf area index increased and then started to decrease due to reduced light penetration into the canopy, reduced photosynthesis and lower leaves abscission. The maximum leaf area index was obtained at tasseling and then decreased over time. Irrespective of hybrids, the maximum leaf area index was related to full irrigated plots. Lower leaf area index at early stages might be due to small cells and lower leaf expansion. Since leaf area is a two-dimensional trait, any increase in leaf growth will result in a progressive increase in leaf area. Cold stress had no significant effect on leaf area index. In all hybrids and in irrigation regimes leaf area index was not affected by cold stress. The maximum leaf area index was observed in single cross 704, 260 and 400, respectively.

Crop growth rate. Over the crop growth period, crop growth rate first increased and then decreased to a negative value. This increase is mainly due to leaf expansion at early growth stages and then a gradual increase in solar radiation absorption (Fig. 3). It is not surprising that increase in leaf area, especially under stress free conditions, increases light absorption and photosynthesis and eventually crop growth rate. By contrast, under stress conditions, crop growth rate would decrease due to leaves senescence an abscission and finally suppressed photosynthesis. The maximum crop growth rate (4.6 g m^{-2} day^{-1}) was observed in single cross 704.

Relative growth rate. The relative growth rate in all maize hybrids grown under cold and water deficit stress conditions showed a descending pattern (Fig. 4). Based on the results, in all stress treatments, relative growth rate decreased with increasing plant age as structural tissues which are not active in photosynthesis were more produced. The higher relative growth rate at early growth stage is mainly due to higher light penetration, higher net photosynthesis and lower leaves shading. It seems that with increasing leaf area duration, leaves photosynthesis and light use efficiency increased which in turn led to higher relative growth rate.

Net assimilation rate. The changes in net assimilation rate in response to cold and water deficit stress in shown in Fig. 5. At early growth stages, increase in net assimilation rate is due to high solar radiation absorption; however over the time with increasing in leaf area and light interception (shading) net assimilation rate would decrease in all hybrids and treatments. Net assimilation rate represents assimilates amount and leaves photosynthesis efficiency. There was a similar pattern in all maize hybrids grown under different irrigation regimes in terms of net assimilation rate. Net assimilation rate first increased and then gradually decreased to the negative value. From the results, severe water deficit stress decreased net assimilation rate more than the other treatments. The maximum difference was observed when leaf area index reached to its maximum.

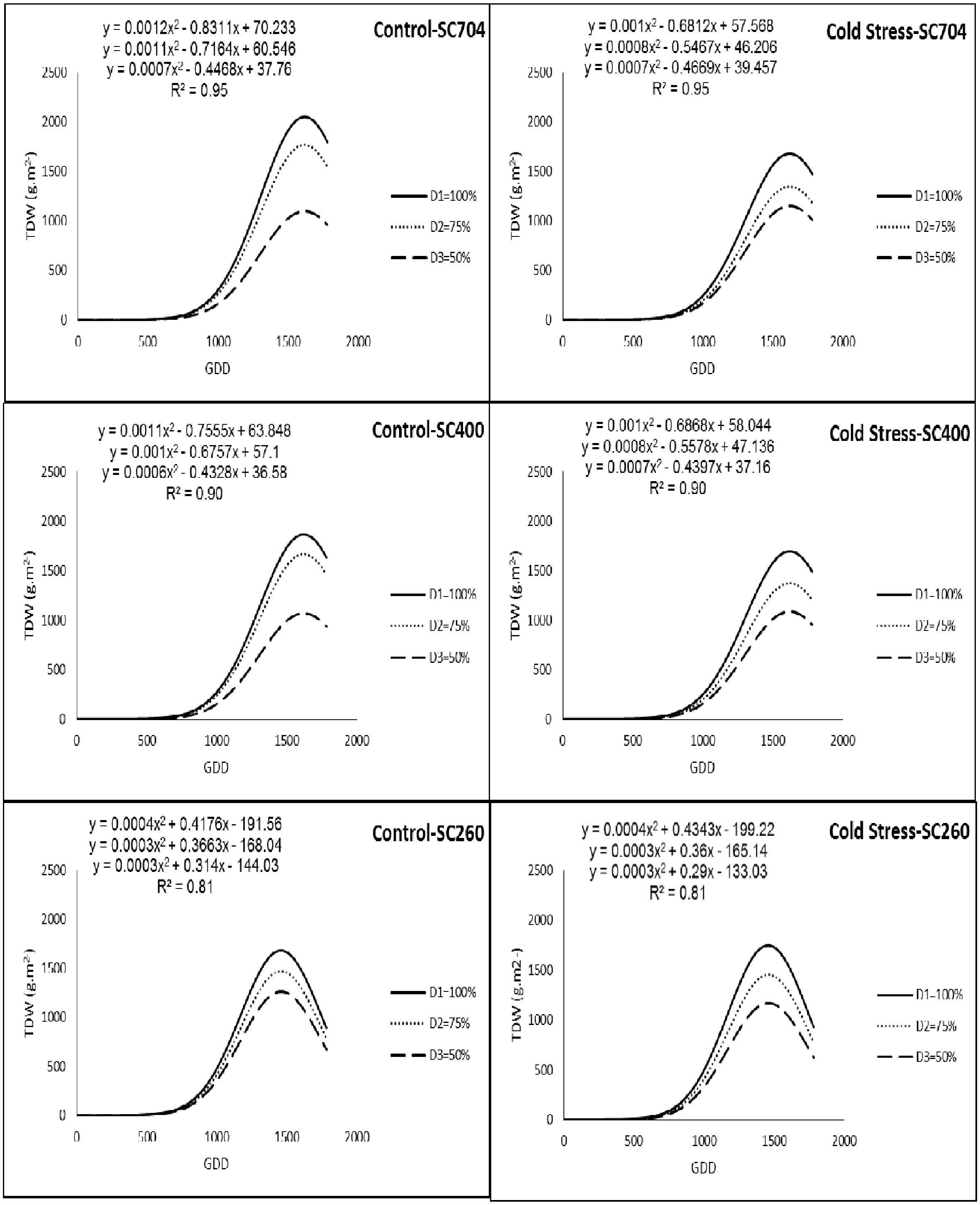

Fig. 1. First year experiment: The effect of irrigation regimes on maize hybrids dry weight under normal and cold stress conditions.

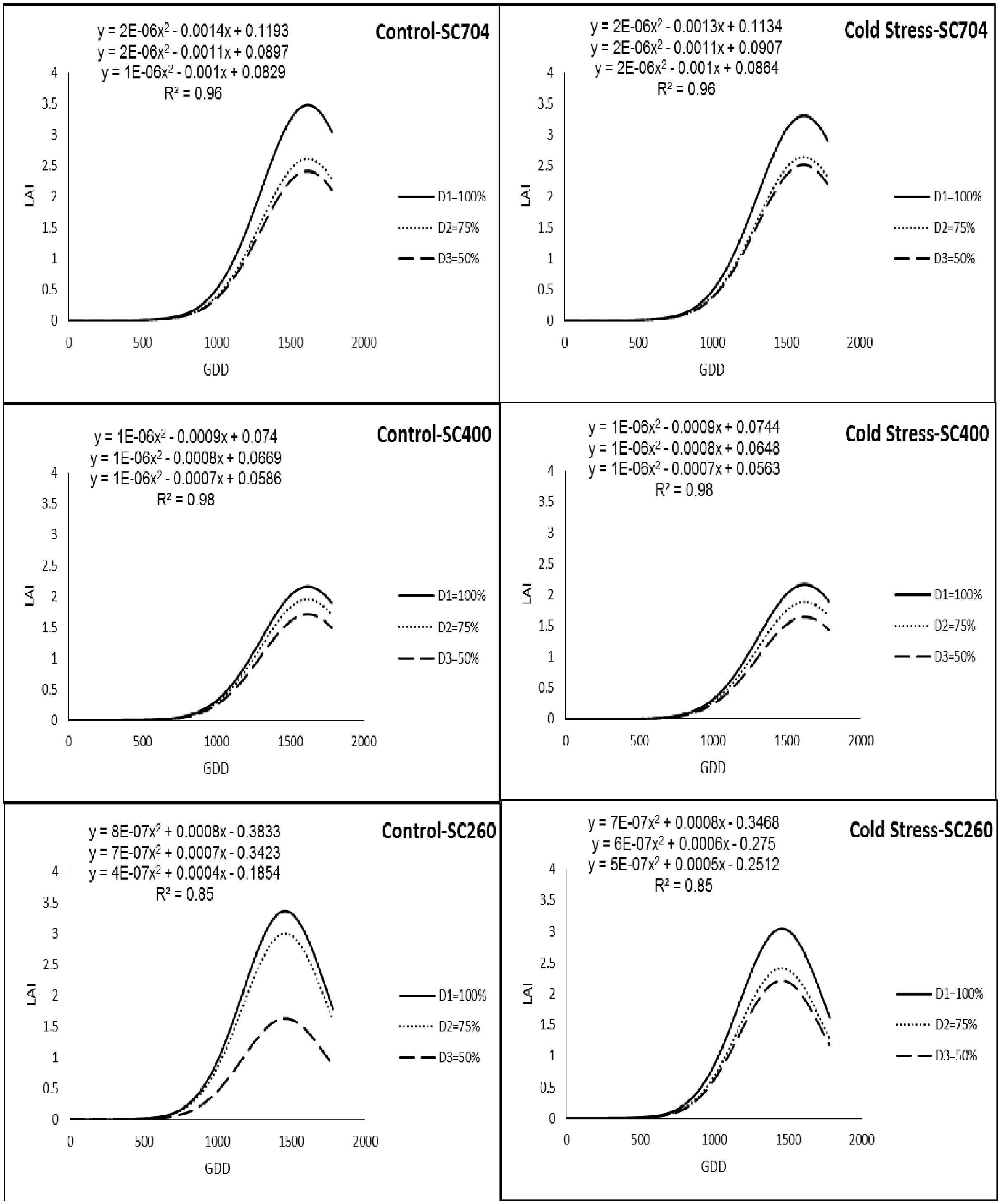

Fig. 2. First year experiment: The effect of irrigation regimes on maize hybrids leaf area index under normal and cold stress conditions.

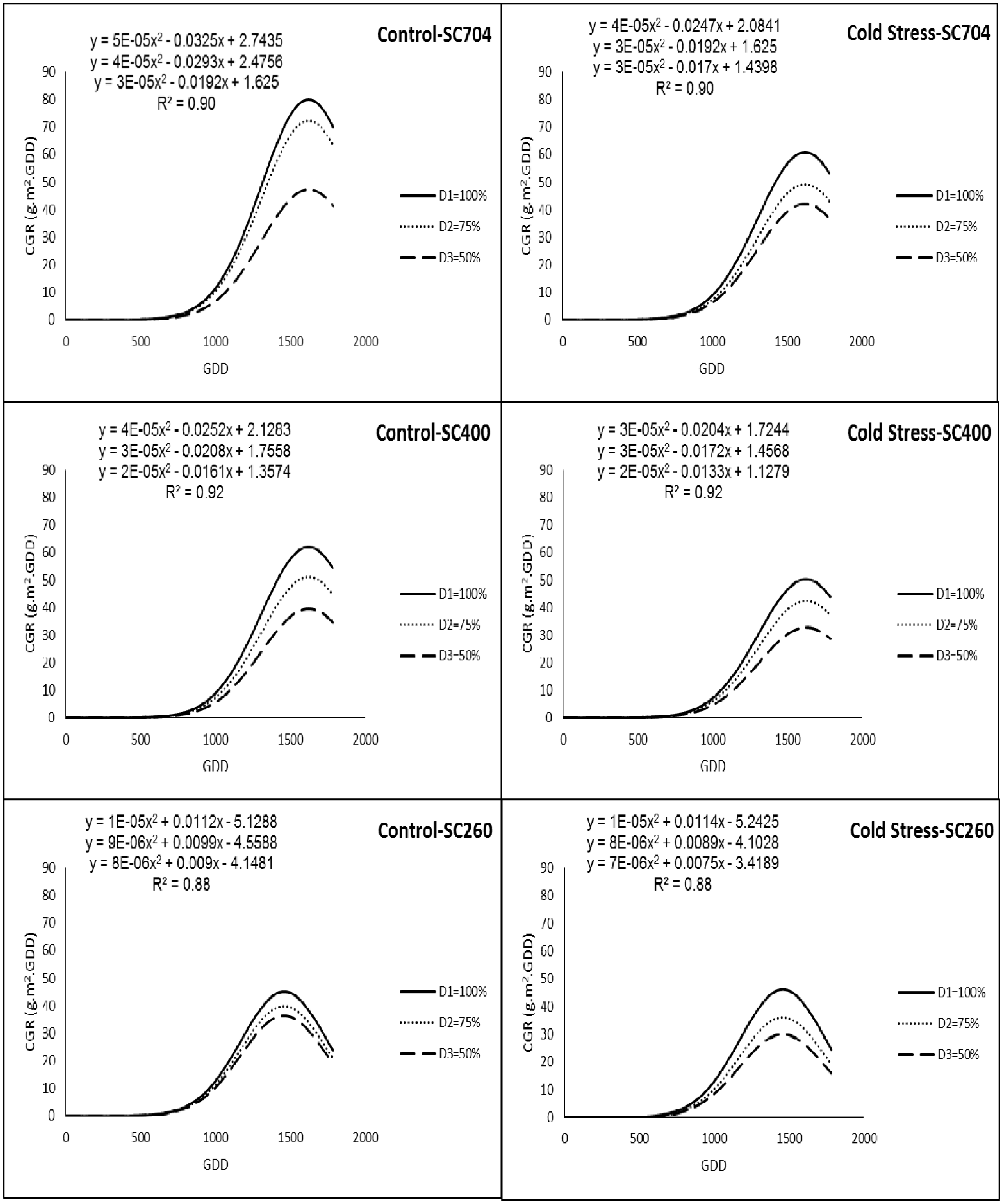

Fig. 3. First year experiment: The effect of irrigation regimes on CGR under normal and cold stress conditions.

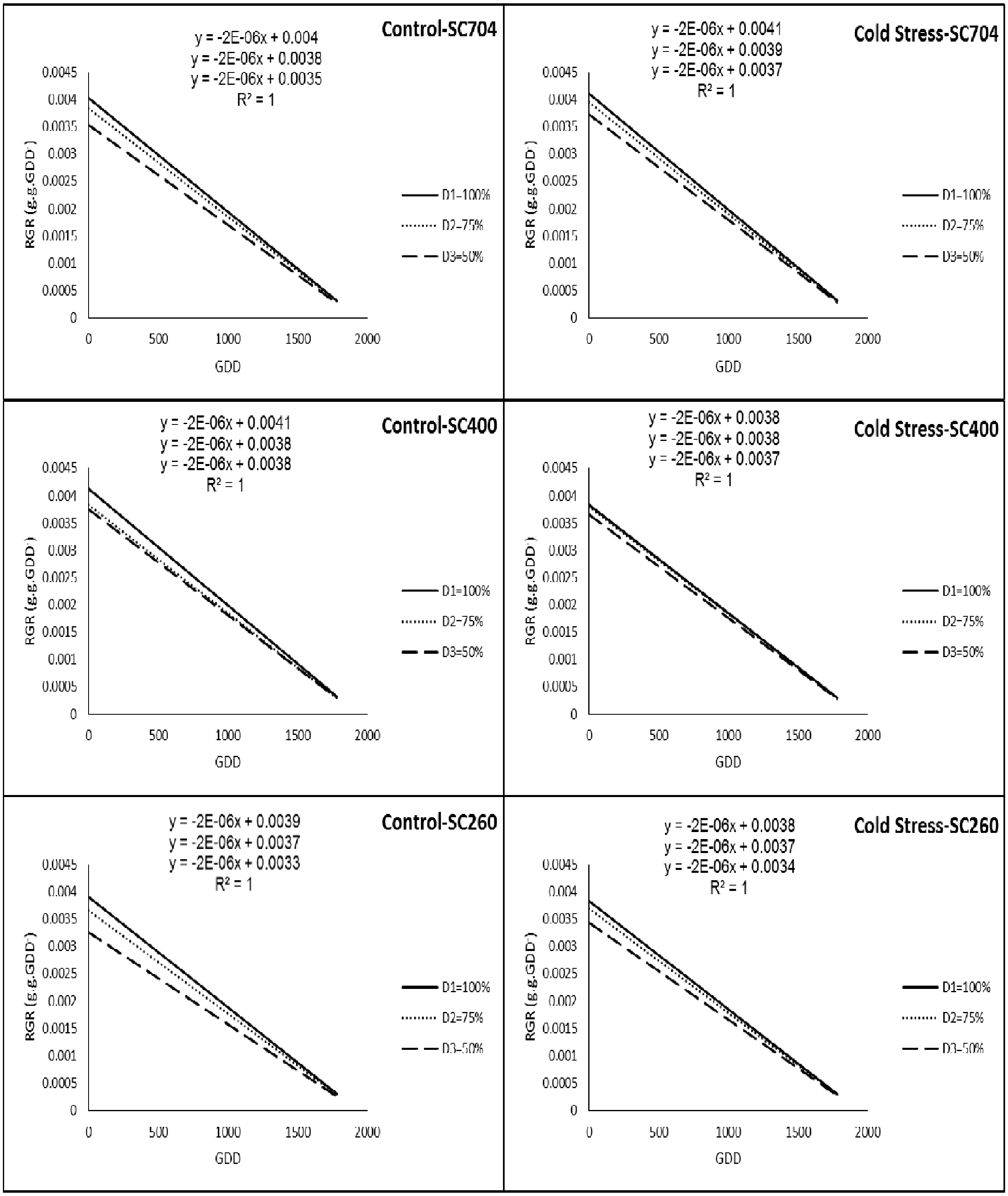

Fig. 4. First year experiment: The effect of irrigation regimes on RGR under normal and cold stress conditions.

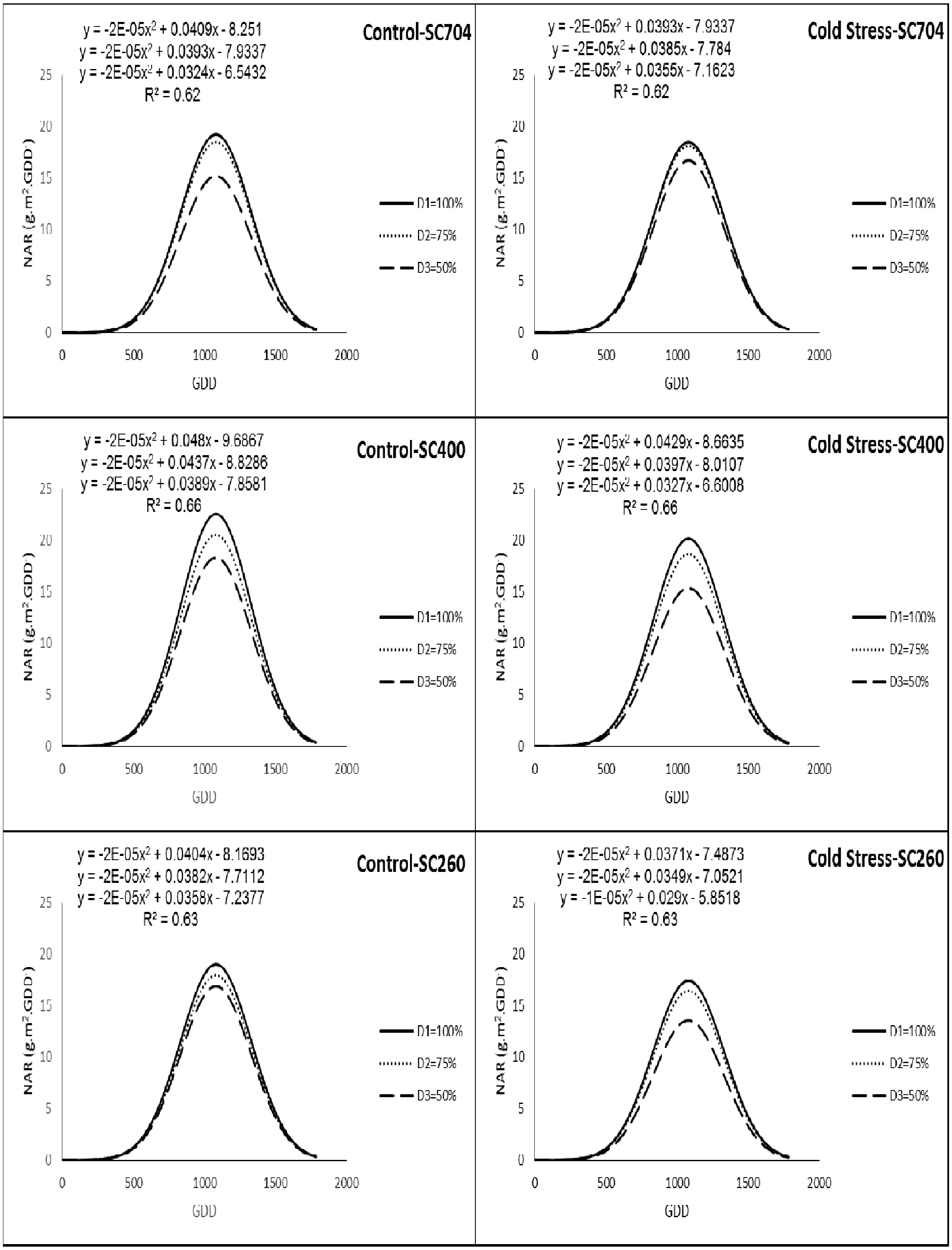

Fig. 5. First year experiment: The effect of irrigation regimes on NAR under normal and cold stress conditions.

This increase was more pronounced in late mature hybrids than early mature hybrids possibly due to faster leaf abscission in stressed late mature hybrids. It seems that the increase in net assimilation rate at early growth stages is due to leaf area limitation and lower intraspecific competition.

B. Second year

Total dry matter. The pattern of dry matter accumulation of crops canopy during growth season is typically characterized by a sigmoid curve so that three distinct phases can be distinguished (i) a period of gradual increase in dry matter during early development, followed by (ii) a period of exponential dry matter accumulation rate, and (iii) a period of declining dry matter accumulation during the final phase of development when green leaf area declines due to leaf senescence and leaf photosynthesis declines due to leaf aging. The results indicated that under both temperature conditions, dry matter accumulation is very low in all irrigation regimes (Fig. 6).

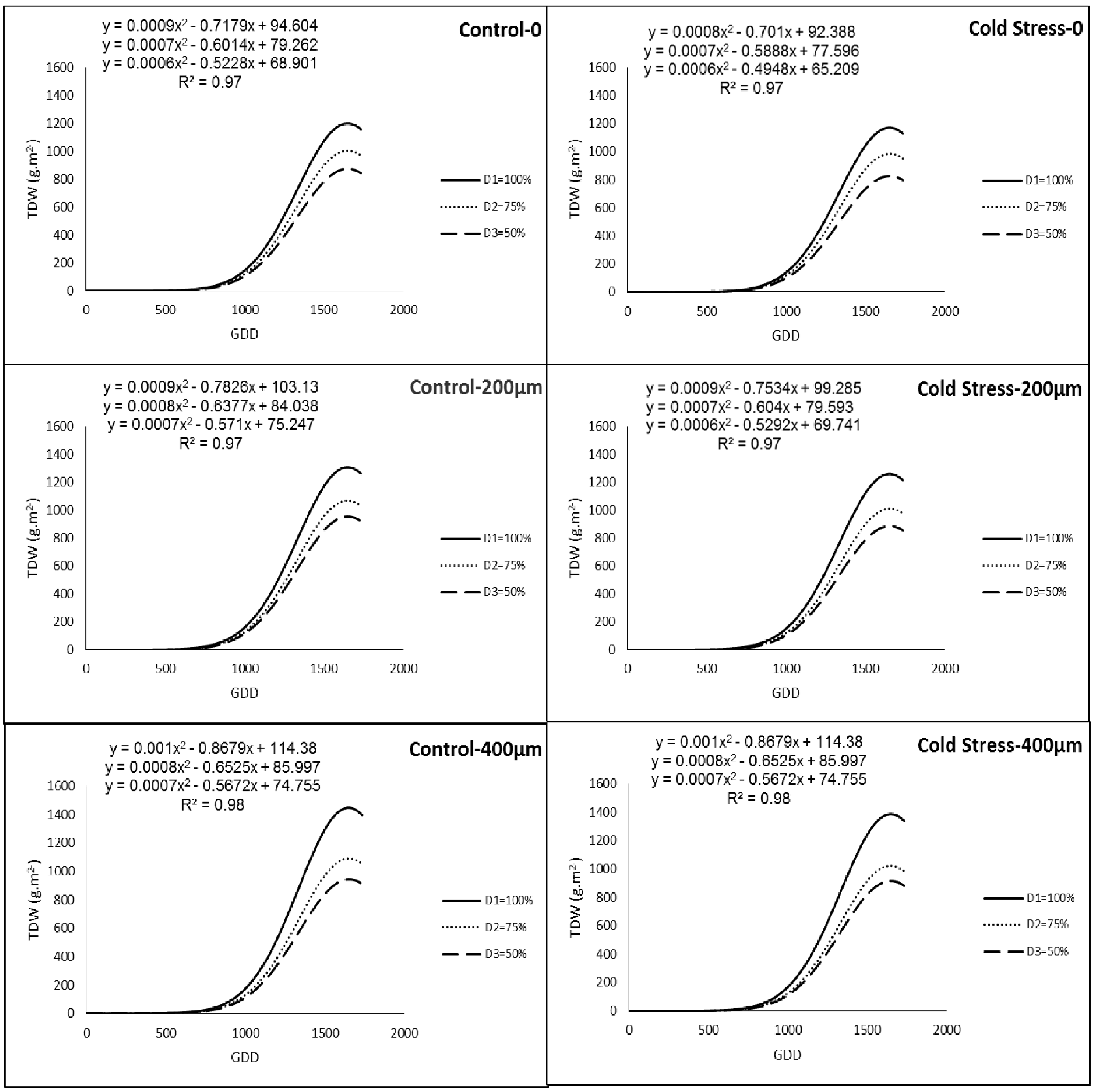

Fig. 6. Second year experiment: The effect of irrigation regimes and salicylic acid on maize hybrids dry weight under normal and cold stress conditions.

At early growth stage, leaves have a low expansion rate. Over the time, dry matter accumulation increases on account of increased leaf area and photosynthesis rate, especially under optimum conditions. In this study, the more dry matter was accumulated in control treatment compared with stress treatments and this increase was more obvious at the time. Salicylic acid foliar application increased plant dry weight. The maximum dry weight (1439 g m^{-2}) was found when 400 mM salicylic acid was applied on the plants.

Leaf area index. Leaf area index over growing period gradually increased from early to late growth stages (Fig. 7).

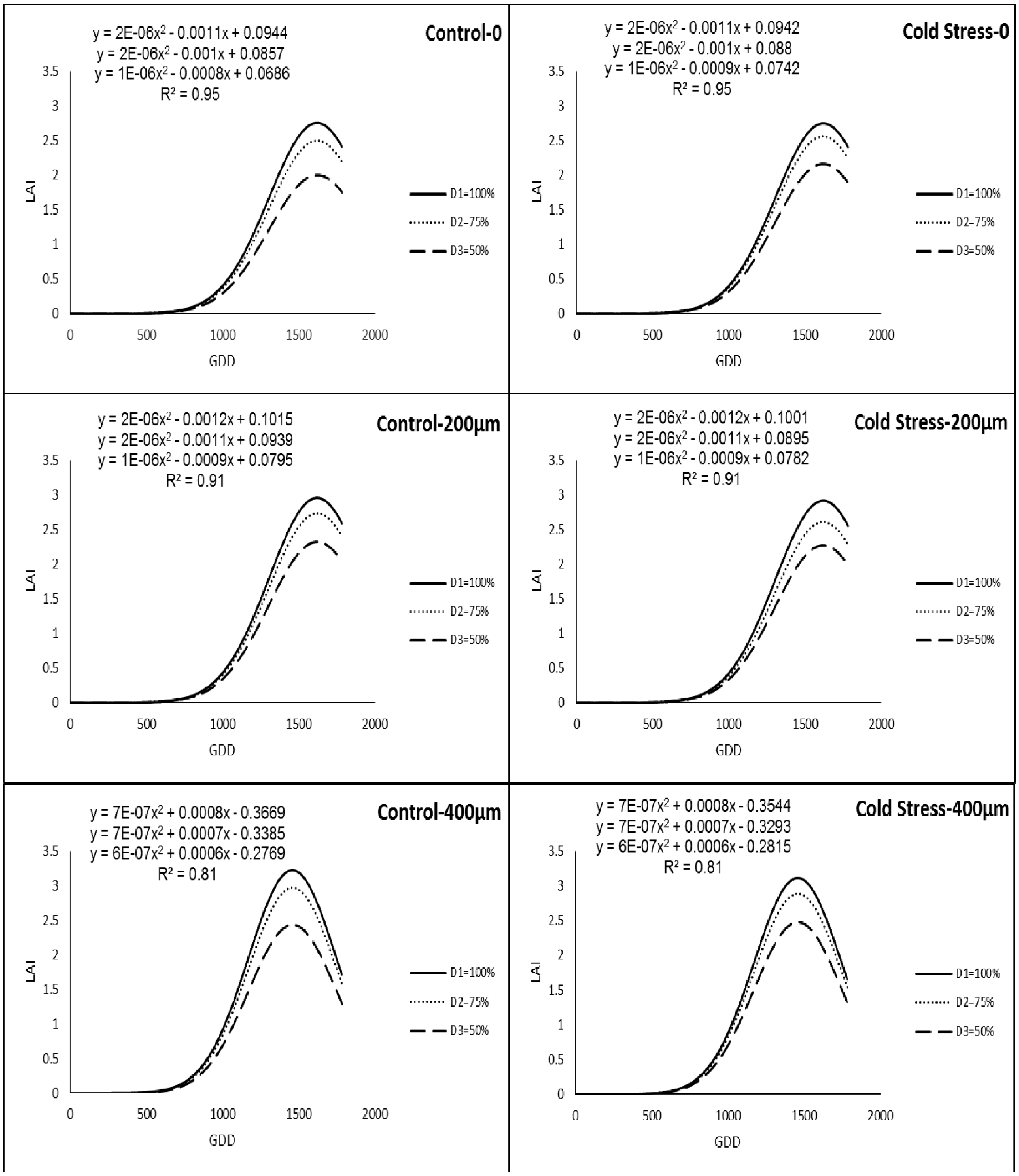

Fig. 7. Second year experiment: The effect of irrigation regimes and salicylic acid on maize leaf area index under normal and cold stress conditions.

Leaf area index decreased with increasing water deficit intensity. Irrespective of cold or water deficit stress, salicylic acid foliar application increased leaf area index. The maximum leaf area index was found when no water stress was applied and plants were sprayed with 400 mM salicylic acid solution.

Crop growth rate. The crop growth rate pattern throughout the season generally increased rapidly to its peak at tasseling stage and then decreased to a negative value (Fig. 8), however, dry matter accumulation continued during crop growth rate descending period.

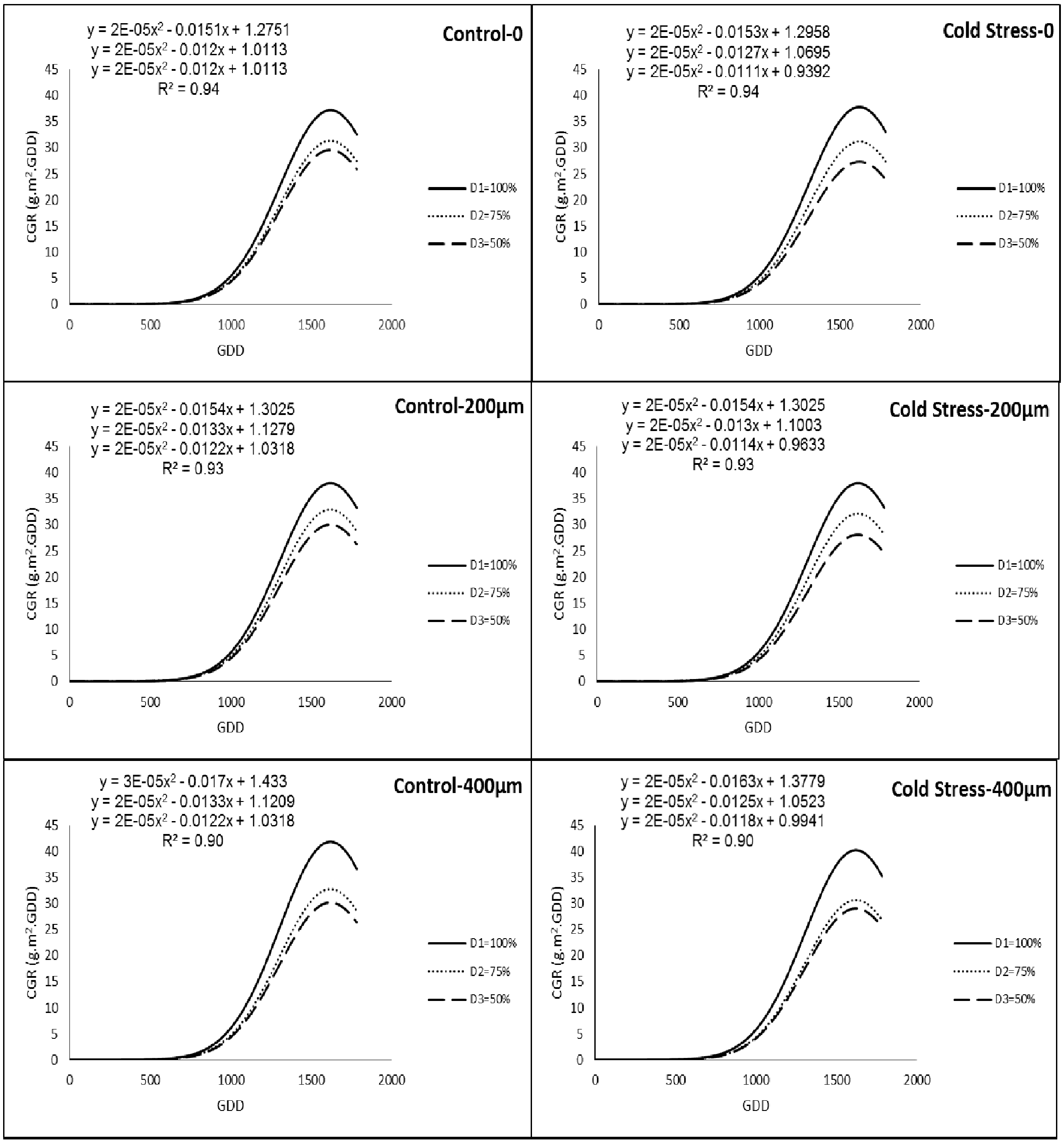

Fig. 8. Second year experiment: The effect of irrigation regimes and salicylic acid on CGR under normal and cold stress conditions.

When crop growth rate is constant dry matter accumulation ends and when crop growth rate is negative total dry matter is decreasing due to leaves and stem degradation. Under both temperature conditions and all irrigation regimes, crop growth rate showed an ascending trend to its maximum followed by a sharp descending trend. The maximum crop growth rate (1.89 g m^{-2} day^{-1}) was obtained when 400 mM salicylic acid was applied on none stressed maize plants. There was no significant difference between cold stressed and non-cold stressed plants.

Relative growth rate. The relative growth rate is defined as the ratio of dry weight change to the achieved growth at a given time and is presented as g g^{-1} day^{-1}. In all treatments, relative growth rate decreased to its minimum and then become negative. As can be seen from Fig. 9, there was no significant difference between treatments in terms of reduction rate.

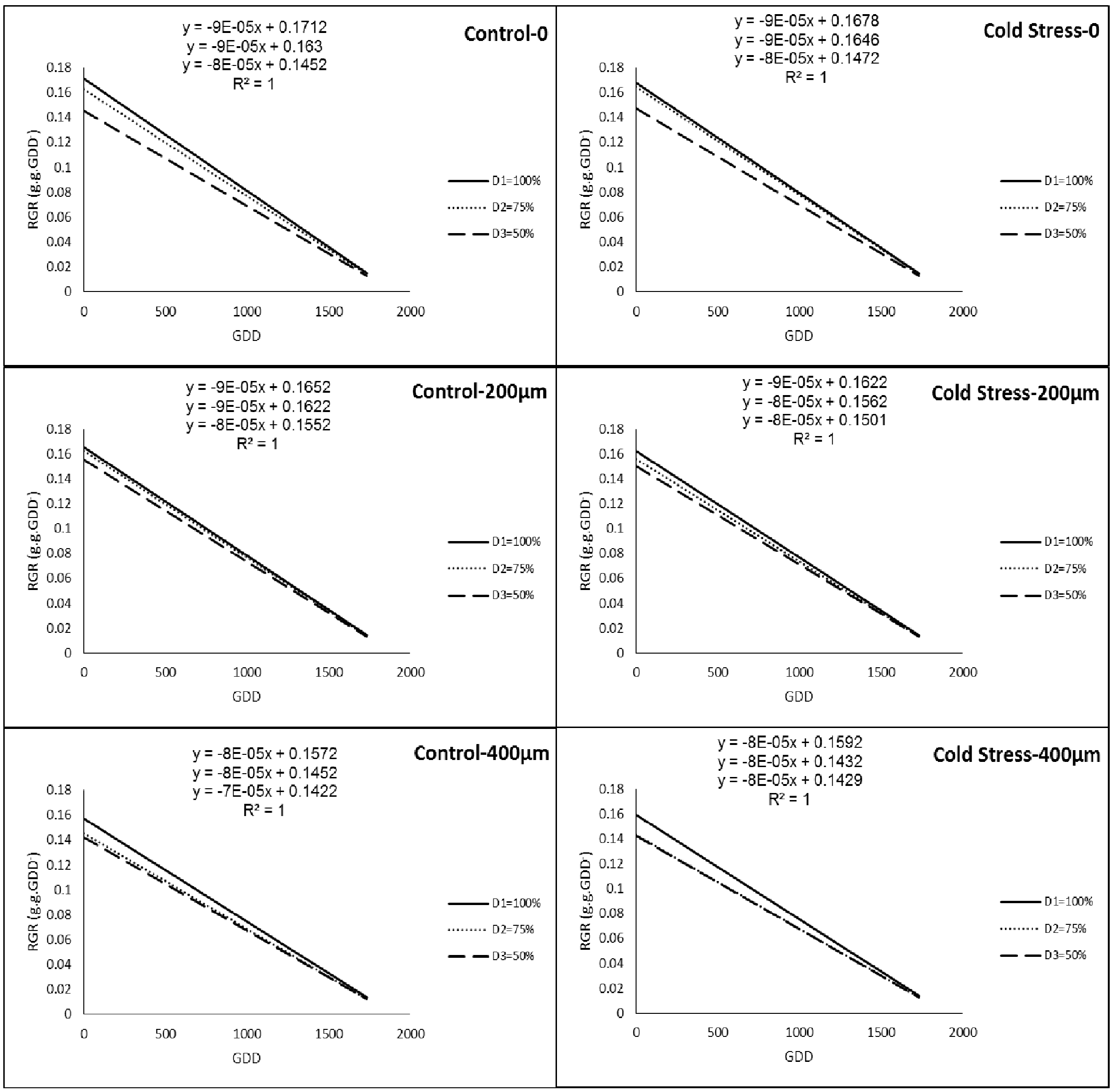

Fig. 9. Second year experiment: The effect of irrigation regimes and salicylic acid on RGR under normal and cold stress conditions.

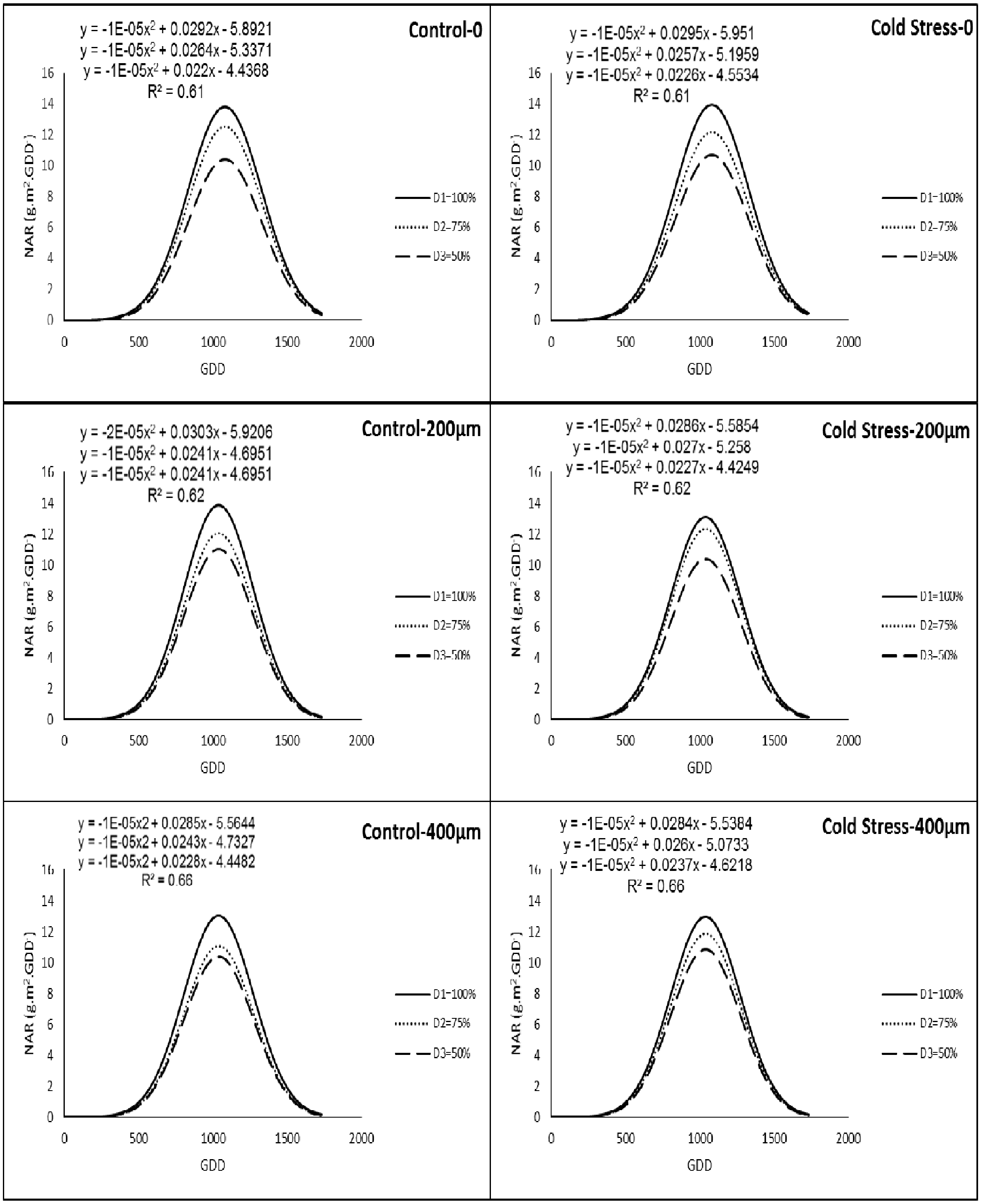

Fig. 10. Second year experiment: The effect of irrigation regimes and salicylic acid on NAR under normal and cold stress conditions.

Net assimilation rate. The maximum net assimilation rate was observed when most of the leaves were exposed to the direct sunlight. In addition to this, increase in leaf area index plays an important role in increasing net assimilation rate. However, if there is further increase in leaf area index, the lowest leaves will be so poorly lit that their respiration will exceed their net photosynthesis. Reduction in net assimilation rate due to increase in leaf area index during growth season was observed in this study. More reduction was found when plants were subjected to severe water deficit stress causing leaf abscission and creating reproductive sinks. Net assimilation rate was similar in cold stressed and non-stressed plants. The net assimilation rate would reach to its maximum if all leaves are exposed to sunlight. Over the time with increasing leaf number and leaf area, upper leaves shade out the lower leaves and cause lower net assimilation rate. Furthermore, leaf aging causes a reduction in photosynthesis which in turn decreases net assimilation rate (Fig. 10).

DISCUSSION

Leaf area index is one of the most important growth indexes determining final yield before flowering. Reduced leaf area index due to water deficit stress is mainly due to reduced assimilates for cell growth and development (Banziger *et al.,* 2000), increase in senescence (Betral *et al.,* 2003). Reduction in leaf area index due to leaf abscission, especially under water deficit stress has been reported by Karimzadeh-asl *et al.,* (2004). Water deficit stress inhibits cell division and expansion and reduces leaf area index (Vyn and Boomsma, 2008). In addition, reduction in leaf area index due to water deficit stress was documented by Azizi *et al.,* (2015). It has been reported that salicylic acid increases maize and soybean leaf area and dry biomass (Khan *et al,* 2003). Salicylic acid affects photosynthesis (Baljani, 2011) and growth rate through increasing leaf area index and improves seed yield. At early growth stages, crop growth rate is at the minimum level due to low leaf area and light use efficiency. With increasing leaf area less light passes through the canopy and then crop growth rate starts to increase (Koliai *et al,* 2013; Tarighaleslami *et al*, 2012). It seems that low leaf area index (leaf senescence and abscission) and reduced solar radiation at the end of growing season are the main reasons for crop growth reduction (Azizian and Sepaskhah, 2014). There is a direct relationship between crop growth rate and absorbed radiation by the leaves. At the beginning and end of growing season less solar radiation is received by the leaves as the canopy is not completely closed; therefore the less dry matter is produced and crop growth rate is at the minimum level. Increase in growth and leaf area increases solar radiation absorption and improves crop growth rate (Habibzadeh *et al.,* 2007). In C_4 plants like maize, crop growth rate is relatively high (30-40 g m^{-2} day^{-1}). According to Ghayor and Karamzade (xxxx) net assimilation rate is not a constant parameter; it is generally a function of the plant age, in other words, as the plant's age increases net assimilation rate will decrease. Environmental stresses such as water deficit stress accelerate net assimilation rate reduction. Reduction in crop growth rate on account of water deficit stress has been previously reported in lentil (Nyari Khamsi *et al.,* 2006) and maize (Terrance *et al.,* 2004). It seems that, at the beginning of the growth season, just before stem elongation stage, the relative growth rate and net assimilation rate are at the highest levels due to more light penetration, less shading and less respiration. However, with closing the canopy over the time, the relative growth rate will decrease and reach to its minimum due to leaf senescence, shading, and lignification, as well as less, assimilates allocation to vegetative and reproductive organs. Similar results have been fuond by other researchers (Ganjali *et al.,* 2000; Oweis *et al,* 2004 and Panahyan, and Jamaati, 2009). Leaf senescence, shading and lignification are known as the main factors in reducing relative growth rate (Tarighaleslami *et al,* 2012). Since structural tissues are not metabolically active, increase in their synthesis reduces relative growth rate (Tarighaleslami *et al,* 2012; Ghayor and Karamzade, 2003). The relative growth rate is decreasing over time. The obtained results are in agreement with those of Terrance *et al.,* (2004) who showed water deficit stress reduces dry matter accumulation and leaf area duration. In addition, Abraham *et al.,* (2008) have shown that limited irrigation decreases leaf area index and crop growth rate in millet. Increase in leaf fresh and dry weight, leaf specific weight and total dry weight in canola on account of salicylic acid application has been reported by Miarsadegh *et al.,* (2011). They have also stated that salicylic acid application could increase cotyledon area and true leaves area under both normal and water deficit stress conditions. The results of the current study mirror Majidian *et al.,* (2002) results who reported that water deficit stress reduces net assimilation rate. Similar results have been found by Wise *et al.,* (1990) who stated that reduction in stomatal conductance is the main reason for reduced net assimilation rate. Increase in leaf area index in canola on account of the salicylic acid application has been previously reported by Miarsadegh (2011).

Anyia and Herzog (2004) have shown that water deficit stress reduces internode number, branch number, SLA and LAR as well as LAI and leaf number in cowpea. Cassto *et al.,* (1997) have reported that leaf area index at seed filling stage determines final seed yield in faba bean grown under water limited conditions. According to Agric *et al.,* (2007) selection based on leaf area index and biomass in maize could improve yield potential under water limited conditions. Application of salicylic before imposing stress could reduce adverse effects of water deficit stress in barley. Reduction in leaf area duration might be due to leaf area index loss and reduced growing period affected by photoperiod and temperature. Low leaf area duration in lower population density is due to reduced leaf area index (Valentinuz, and Tollenar, 2004; Subedi and Ma, 2005).

CONCLUSION

Early seed sowing and cold stress showed no negative effects on growth parameters. Early sowing improved growth parameters under cold stress conditions. Generally, we can conclude that water deficit stress, especially during reproductive stage, had negative effects on growth parameters and finally seed yield. Reduction in leaf number and leaf area due to water deficit stress is a result of reduced cell division. In general, according to the results, it seems that application of salicylic acid could improve growth parameters in maize and increase water deficit tolerance.

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