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SEED PRIMING WITH SALICYLIC ACID ENHANCED GAS EXCHANGES PARAMETERS AND BIOLOGICAL YIELD OF WHEAT UNDER LATE SOWING DATE

SUMMARY
This experiment was done to evaluate the effect of two planting dates and salicylic acid (SA) on wheat photosynthesis. Wheat seeds, cv. Alvand, primed with SA (0, 400, 800, 1200, 1600, 2000 and 2400 μM) at two planting dates (recommended planting date, 23 October, and late planting date, 22 November). Gas exchange parameters were measured in three growth stages (tillering, heading and grain filling). The highest and lowest rate of photosynthesis ($P_N$), stomatal conductance ($g_s$) and transpiration rate ($E$) of plants were observed in heading and grain filling stages, respectively. Seed pretreatment with SA enhanced photosynthetic parameters and carboxylation efficiency (CE), but, intercellular CO$_2$ concentration and water use efficiency (WUE) reduced by application of SA. It seems that application of SA had more effects on $g_s$ and $E$ than $P_N$. Among growth stages, the highest value of WUE was found in tillering and lowest in heading stage. Priming with SA compensated late sowing effects on plants $P_N$. Chlorophyll content, chlorophyll a/b ratio and CCI values significantly increased in SA treated plants. Results show that priming with SA may reduce ameliorative effects of late sowing on wheat plant biomass production. Among SA concentrations, 1200 μM had highest value in both planting dates.

Keywords: Carboxylation efficiency, Chlorophyll content, Photosynthesis rate, Water use efficiency.

INTRODUCTION
Photosynthesis and related gas exchange parameters influenced by many internal and external factors. For example, it is reported leaf ontogeny, heterophylly and position (Hejnak et al., 2014), age (Wang et al., 2014), seasonal changes and conditions (Ribeiro et al., 2009), sink effect (Nebauer et al., 2011) have considerable effects on photosynthesis rate and its regulation. Level of leaf development and/or morphological and anatomical stage of plant may influence photosynthesis rate (Hejnak et al., 2014). Also, environmental history of leaves affect their photosynthetic development (Fitter and Hey, 2012).

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Sowing date is a key factor on plants productivity potential and has deep effect on crop yield. It can by influence on plant tissues age change photosynthetic capacity of plants.

Salicylic acid (SA) is an endogenous growth regulator with phenolic structure, which participates in the regulation of different physiological and biochemical processes in plants (Raskin, 1992) and acts as an important signaling molecule (Nazar et al. 2011). SA, might play a role in $g_s$ (Janda et al., 2014), photosynthesis (Fariduddin et al. 2003), and stomatal closure (Poor and Tari, 2012). Nazar et al. (2011) reported in mungbean SA increased photosynthesis under normal condition and alleviated salt effects on photosynthesis. These protective effects may be related with nitrogen and sulfur assimilation metabolism. Spraying of maize plants with SA led to increase photosynthesis, pigment content and growth rate (Khodary, 2004).

At many parts of Iran, because of unfavorable weather condition, rotation of wheat after late potato or maize cultivars and/or large planting areas, planting date may delay until late of autumn and these situations leads to weakly growth of seedlings or fail in establishment by cold stress damage. On the other hand, it is possible later growth and photosynthesis of plants after winter affected by pervious growth stages and growth history of plants. The aim of our experiment was to study the effects of two different sowing dates on gas exchange parameters and biological yield of wheat and possibility of ameliorative effects of seed priming by SA on these parameters.

**MATERIAL AND METHODS**

Seeds of wheat (*Triticum aestivum* L., cv Alvand) obtained from seed bank of faculty of Agriculture, University of Zanjan. Seed moisture content was 8.54% (based on dry weight). For all treatments, selected healthy seeds were used in same numbers.

**Salicylic acid seed treatments**

For seed priming, SA solutions prepared in six levels, including 400, 800, 1200, 1600, 2000 and 2400 µM. For each treatment SA powder (Merck, Darmstadt. Germany) weighted separately and solved in 5 cc ethanol and shaken well. Then solution added to 3 litter distilled water. The ratio of seed to solute was 1:5 (based on weight). Seeds submerged for 12 hours at 4 °C. Then, seeds exposed to airflow and air-dried. Non primed seeds, were used as control treatment.

**Planting and cultivation:**

Seeds planted in two planting dates: 23 October (as conventional planting date in Zanjan province) and 22 November (as late planting date) of 2010 in research station of University of Zanjan (36°40’ N; 48°24’ E and 1610 m from sea level). In general, Iran has arid and semi-arid climate and the major precipitation occur from October to June (Table 1). The coldest month mainly occurs in January and cold weather and frost happen mid to end of November to March (Alijani, 2006). The day after planting plots irrigated and irrigation continued
Seed priming with salicylic acid enhanced gas exchanges parameters...

until frizziness temperature was appeared. In spring from 4th of May irrigation again started normally each week. According to soil analysis 40 kg/ha phosphate in form of phosphate ammonia before planting and 80 kg/ha nitrogen in form of urea in two times (after seedlings emergence and before stem elongation) were added to field.

Table 1. Mean temperature of 10 years (from 1999-2009) and the experiment year (2009-10) of Zanjan region.

| Months  | Max | Min | Max | Min |
|---------|-----|-----|-----|-----|
| October | 27.2| -1.6| 26.8| 0.3 |
| November| 18.8| -7  | 18.4| -8  |
| December| 11.8| -8.4| 12.4| -13.9|
| January | 14.4| -15.4| 10.5| -17.3|
| February| 17.4| -12 | 12.8| -11.8|
| March   | 25.8| -4.2| 20.3| -7.6 |
| April   | 23  | 0.2 | 25  | -2.6 |
| May     | 29  | 1.2 | 29  | 2.1  |
| June    | 35.6| 9   | 34.4| 5.9  |
| July    | 40  | 10  | 36.1| 10.1 |

**Gas exchange**

Gas exchange parameters in three growth stages (tillering, heading and grain filling) were recorded. Photosynthesis rate ($P_N$), stomatal conductance ($g_s$), transpiration rate ($E$) and intercellular CO$_2$ concentration ($C_i$) were measured using a portable open-system infrared gas analyzer (LCi, ADC Bioscientific Ltd., Hoddesdon, UK).

All measurements were done in 10-12 a.m. and light intensity equivalent to 1200-1800 μmol photons.m$^{-2}$. It is reported that $g_s$ during 10 am to 1 pm no significant changes (Clark and Mc ciag, 1982) and also at this light intensity $g_s$ reaches to a maximum. Before measuring, apparatus started for 10 minutes. For measuring gas exchange parameters, same leaves of plants in each treatment placed in chamber glass clamp of apparatus. Data recorded after 45 seconds as the chamber conditions receive as stable state.

Photosynthetic water use efficiency (WUE) and Carboxylation efficiency (CE) also was calculated based on the following formula (Ashraf et al; 2002):

Photosynthetic water use efficiency ($WUE_b$) = $P_N / E$

Carboxylation efficiency (CE) = $P_N / C_i$

**Chlorophyll content index (CCI)**

Chlorophyll content index was measured by a chlorophyll meter handheld device (CCM-200 ADC, UK) in all three stages from 10 randomly selected plants’. Middle part of same leaves was used for this reason. In tillering fourth or fifth leaf was measured and in two later growths stages the flag leaves were measured.
Chlorophyll a, b and total:
Chlorophyll content of flag leaf in anthesis stage determined by method which described by Meidner (1984). The statistical analysis was done by using software MSTATC and SPSS. Means comparison was done by Duncan multiple test.

RESULTS AND DISCUSSION
Photosynthesis rate ($P_N$)
In both planting date and in all three growth stages priming significantly increased $P_N$ rate. Except to grain filling stage, in tillering and heading stages early planted wheat had higher $P_N$ compared to late plated wheat (Table 2, 3 and 4). The highest $P_N$ in both planting date was observed in heading stage, then in tillering stage. In both planting dates, a decline was found in $P_N$ in grain filling stage compared to heading stage (Table 3 and 4). In addition, at grain filling stage, all prim treatments in late planting had higher $P_N$ compared to conventional planting date. It seems that, plants in the late planting treatment had younger tissues than conventional planting date. Also, it seems that, in grain filling compared to heading and tillering stages leaves was matured and more aged and therefore, had lower capacity in photosynthesis. In addition, it is possible in heading and pollination stage there was a higher demand for photoassimilates and may be it is a reason for the highest $P_N$ in this stage. As mentioned above, priming with SA leads to increasing $P_N$. Among priming treatments, priming with 1200 µM concentration, in both planting date and in all three growth stages had the highest rate of photosynthesis (Table 2, 3 and 4).

Enhancement activity of carbonic anhydrase in leaves of mustard (Fariduddin et al. 2003) and Rubisco in maize (Khodary, 2004) by application of SA was reported. Also, protection of the photosynthetic apparatus has also been reported in SA treated tomato plants (Poor et al., 2011). On the other hand, higher concentrations of SA may have prevention effects on photosynthesis (Janda et al., 2014). Our results show that in concentrations over 1200 µM a decline observed in $P_N$.

Stomatal conductivity ($g_s$)
Seed priming increased $g_s$ in three stages and in both planting dates. Also, it compensated the reduction in $g_s$ in late planting in all growth stages. $g_s$ in both planting and in the heading stage reached highest value in 1200 µM SA compared to other SA treatments (Table 2, 3 and 4). In comparison of two planting dates, $g_s$ in late planting had higher value than conventional planting date in grain filling stage. Among growth stages the lowest $g_s$ were observed in grain filling and highest in heading stage. The high ratio of $g_s$ in heading compared to grain filling and tillering may be due to young age of leaves and demand of atmosphere for transpiration, respectively.

In contrast, in tillering stage as environment was cool then two other stages the $g_s$ showed lower amounts. It is reported exogenous application of SA in wheat promoted growth and yield which associated with increased
photosynthesis capacity and $g_s$ (Arfan et al. 2007).

**Transpiration rate ($E$)**

The highest $E$ was observed in the heading and the lowest in tillering and grain filling stages. Priming increased $E$ in all different growth stages. Among priming treatments, 1200 µM had the most effects on $E$ in both planting dates and all growth stages. In general, priming by SA improved the $E$ values and enhanced its values compared to control treatments (Table 2, 3 and 4). Higher rate of transpiration may be related to increasing root development or efficient uptake of water by increasing root length and density which reported by Sandoval-Yepiz (2004) and Abdolahi and Shekari (2013). The lower value of $E$ in tillering and grain filling stages may be due to cool temperature in early spring; and maturation of leaves, and therefore, reduction in capacity of leaves transpiration from aged plants, respectively.

**Intercellular CO2 concentration ($C_i$)**

$C_i$ was decreased by application of SA in three stages and two planting date and control treatments had higher $C_i$ values. Among three growth stages the lowest $C_i$ was found in heading stage and two other stages had higher values than mentioned stage (Table 2, 3 and 4). In general, 1200 µM of SA showed the lowest rate in all treatments expect tillering stage. In tillering stage 400 and 800 µM and in grain filling 400 µM of SA had not significant differences with control treatments. In tillering stage 2000 and 1600 µM SA in conventional and late planting had lowest $C_i$ respectively (Table 2, 3 and 4). Lower rate of $C_i$ in priming treatments than control treatments may due to higher CE or higher performance in assimilation of CO2 in photosynthesis process. It was reported foliar spraying of 0.5-2.5 mmol.L$^{-1}$ of SA on cucumber seedlings before the low temperature stress increased the leaf $P_N$, $g_s$, $E$, Φ PSII, Fv/Fm, while decreased the $C_i$ (Liu et al., 2009).

**Carboxylation efficiency (CE)**

Priming significantly increased CE in all growth stages and two planting dates (Table 2, 3 and 4). The highest value of CE was observed in heading stage and conventional planting date and the lowest was observed in grain filling stage and conventional planting date. Since, the values of $P_N$ were higher and $C_i$ was lower in this stage, it is reasonably highest amounts of CE found in this stage. In heading stage, priming in highest values improved CE to 42% and 26% on the first and second planting date compared to control treatments, respectively. At all phenological stages, 1200 µM treatment, had the highest CE rate. High CE in priming treatments suggests more effective assimilation of carbon in these treatments relative to control treatments. Zhen et al. (2010) reported Chrysanthemum plants treatment with ASA increased CE, so caused reduction in $C_i$ under low temperature stress with lower light intensity. They suggested that more tolerance to cold stress correlated with higher values of CE, $g_s$ and $P_N$.

**Photosynthetic water use efficiency (WUE)**

The lowest rate of WUE was observed in heading stage and the highest was observed in tillering stage (table 2, 3 and 4).
Table 2. Effect of priming by salicylic acid (SA) on, net photosynthetic rate (PN), stomatal conductance (gs), Transpiration Rate (E), intercellular CO$_2$ concentration (Ci), Carboxylation efficiency (CE), and photosynthetic water use efficiency (WUE) in tillering stage in wheat plants.

| Planting date | Priming [µM] | $P_N$ [µmol (CO$_2$) m$^{-2}$ s$^{-1}$] | $g_s$ [mmol m$^{-2}$ s$^{-1}$] | $E$ [mmol (H$_2$O) m$^{-2}$ s$^{-1}$] | $Ci$ [µmol(CO$_2$) mol(air)$^{-1}$] | CE [µmol (CO$_2$) mmol$^{-1}$ (H$_2$O)] | WUE [µmol (CO$_2$) mmol$^{-1}$ (H$_2$O)] |
|---------------|--------------|---------------------------------------|-------------------------------|-----------------------------------|-----------------------------------|---------------------------------------|---------------------------------------|
| **Control**   | 13.472$^{bc}$ | 175$^d$                               | 3.572$^h$                     | 233.5$^a$                         | 57.82$^{efg}$                     | 3.77$^a$                              |
| **400**       | 15.403$^{abc}$ | 245$^c$                               | 4.375$^{de}$                  | 233.5$^a$                         | 65.98$^{bde}$                     | 3.52$^{abc}$                          |
| **800**       | 16.712$^{abc}$ | 292.5$^b$                             | 5.463$^a$                     | 232.5$^a$                         | 83.04$^a$                         | 3.06$^{abc}$                          |
| **1200**      | 18.155$^a$    | 325$^a$                               | 5.84$^a$                      | 216.75$^b$                        | 83.175$^a$                        | 3.396$^{abc}$                         |
| **1600**      | 16.862$^a$    | 250$^c$                               | 4.927$^b$                     | 216.5$^b$                         | 69.183$^{bc}$                     | 3.058$^{bc}$                          |
| **2000**      | 15.473$^{bc}$ | 202.5$^d$                             | 4.33$^{de}$                   | 185.833$^f$                       | 55.763$^{fg}$                     | 3.532$^{abc}$                         |
| **2400**      | 15.028$^{bc}$ | 200$^d$                               | 4.232$^{efg}$                 | 200.75$^{cd}$                     | 52.403$^g$                        | 3.107$^{abc}$                         |
| **Late**      | 12.335$^{bc}$ | 175$^d$                               | 3.742$^{gh}$                  | 234.75$^a$                        | 63.675$^{dde}$                    | 3.512$^{abc}$                         |
| **planting**  | 14.403$^{abc}$ | 181.667$^d$                           | 4.239$^{efg}$                 | 218.5$^b$                         | 64.983$^{dde}$                    | 3.462$^{abc}$                         |
| **400**       | 15.048$^{abc}$ | 235$^c$                               | 4.259$^{ef}$                  | 206.75$^c$                        | 70.745$^{bc}$                     | 3.141$^{abc}$                         |
| **800**       | 17.092$^{ab}$ | 252.5$^c$                             | 4.852$^{bc}$                  | 204$^{cd}$                        | 73.35$^b$                         | 3.572$^{abc}$                         |
| **1200**      | 14.955$^{bc}$ | 237.5$^e$                             | 4.782$^{bde}$                 | 192.25$^e$                        | 65.983$^{bcd}$                    | 2.888$^c$                            |
| **1600**      | 13.523$^c$    | 202.5$^d$                             | 4.573$^{bcde}$                | 198.25$^{de}$                     | 61.46$^{def}$                     | 3.789$^{ab}$                          |
| **2000**      | 12.35$^{bc}$  | 140$^e$                               | 3.743$^{fgh}$                 | 204.75$^{cd}$                     | 54.62$^{fg}$                      | 3.305$^{abc}$                         |

*ANOVA*

| date          | **  | ***  | ***  | ***  | n.s. | n.s. |
|---------------|-----|------|------|------|------|------|
| Priming       | n.s.| ***  | ***  | ***  | ***  | *   |
| date×priming  | **  | ***  | ***  | ***  | ***  | *   |

*Within each column, different letters indicate significant differences at P ≤ 0.05 (Duncan test). n.s., *, ** and *** indicate non-significant or significant differences at P, 0.05, 0.01 or 0.001, respectively.
Table 3. Effect of priming by salicylic acid (SA) on, net photosynthetic rate (PN), stomatal conductance (gs), Transpiration Rate (E), intercellular CO$_2$ concentration (Ci), Carboxylation efficiency (CE), and photosynthetic water use efficiency (WUE) in heading stage in wheat plants.

| Planting date | Priming  [µM] | $P_N$  [µmol (CO$_2$) m$^{-2}$s$^{-1}$] | g$_s$  [µmol m$^{-2}$s$^{-1}$] | $E$  [mmol (H$_2$O) m$^{-2}$s$^{-1}$] | Ci  [µmol (CO$_2$) mol (air)$^{-1}$] | CE  [µmol (CO$_2$) m$^{-2}$s$^{-1}$] | WUE  [µmol (CO$_2$) mmol$^{-1}$ (H$_2$O)] |
|---------------|---------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Stress period |               |                                 |                 |                 |                 |                 |                 |
| Control       | 17.758$^{gh}$ | 345$^{ef}$                      | 8.11$^{cde}$    | 238.5$^{ab}$    | 78.79$^{hi}$    | 1.945$^{c}$     |                 |
| 400           | 21.535$^{bc}$ | 382.5$^{de}$                    | 8.565$^{gbede}$ | 225.733$^{cd}$  | 87.485$^{defg}$ | 2.259$^{abc}$   |                 |
| 800           | 22.187$^{ab}$ | 420$^{cd}$                      | 9.77$^{ab}$     | 216.25$^{de}$   | 99.410$^{c}$    | 2.517$^{a}$     |                 |
| 1200          | 23.553$^a$   | 537.5$^b$                       | 10.225$^a$      | 209.5$^e$       | 111.79$^a$      | 2.001$^c$       |                 |
| 1600          | 20.395$^{bcd}$ | 502.5$^b$                     | 9.68$^{ab}$     | 210.5$^e$       | 108.79$^{ab}$   | 2.271$^{abc}$   |                 |
| 2000          | 19.897$^{cd}$ | 420$^{cd}$                      | 8.735$^{bcde}$  | 225.25$^{cd}$   | 103.09$^{bc}$   | 2.111$^{bc}$    |                 |
| 2400          | 19.077$^{defg}$ | 332.5$^{ef}$                  | 7.910$^{de}$    | 234.233$^{abc}$ | 84.095$^{efgh}$ | 2.305$^{abc}$   |                 |
| Late planting |               |                                 |                 |                 |                 |                 |                 |
| Control       | 17.052$^h$   | 307.5$^f$                       | 7.555$^e$       | 242.733$^a$     | 75.120$^i$      | 2.163$^{abc}$   |                 |
| 400           | 17.967$^{fgh}$ | 332.5$^{ef}$                  | 8.978$^{gabcd}$ | 218$^{de}$      | 78.226$^{hi}$   | 2.165$^{abc}$   |                 |
| 800           | 19.025$^{defg}$ | 465$^{bc}$                     | 9.058$^{abcd}$  | 216.25$^{de}$   | 89.358$^{def}$  | 2.140$^{bc}$    |                 |
| 1200          | 20.672$^{bcd}$ | 622.5$^a$                       | 9.58$^{ab}$     | 210.25$^c$      | 94.82$^{cd}$    | 2.278$^{abc}$   |                 |
| 1600          | 19.69$^{def}$ | 397.5$^{cde}$                   | 9.207$^{abc}$   | 217.25$^{de}$   | 90.58$^{de}$    | 2.135$^{bc}$    |                 |
| 2000          | 18.150$^{efgh}$ | 355$^{def}$                  | 8.497$^{bcde}$  | 218$^{de}$      | 81.025$^{fghi}$ | 2.419$^{ab}$    |                 |
| 2400          | 17.453$^{gh}$ | 327.5$^{ef}$                    | 7.82$^{de}$     | 230.66$^{7bc}$  | 80.36$^{ghi}$   | 2.232$^{abc}$   |                 |

ANOVA

| Date          | *** | n.s. | n.s. | n.s. | *** | n.s. |
|---------------|-----|------|------|------|-----|------|
| Priming       | *** | ***  | *    | ***  | *** | *    |
| date × priming| *** | ***  | *    | ***  | *** | *    |

* Within each column, different letters indicate significant differences at P ≤ 0.05 (Duncan test). n.s., *, ** and *** indicate non-significant or significant differences at P, 0.05, 0.01 or 0.001, respectively.
Table 4. Effect of priming by salicylic acid (SA) on, net photosynthetic rate (PN), stomatal conductance (gs), Transpiration Rate (E), intercellular CO2 concentration (Ci), Carboxylation efficiency (CE), and photosynthetic water use efficiency (WUE) in grain filling stage in wheat plants.

| Planting date | Priming [µM] | PN [µmol (CO2) m⁻² s⁻¹] | gₜ [mmol m⁻² s⁻¹] | E [mmol (H₂O) m⁻² s⁻¹] | Ci [µmol (CO2) mol(air)⁻¹] | CE [mmol (CO2) m⁻² s⁻¹] | WUE [µmol (CO₂) mmol⁻¹ (H₂O)] |
|---------------|--------------|--------------------------|-------------------|-------------------------|---------------------------|--------------------------|-----------------------------|
|               | **           | **                       | **                | **                      | **                        | **                       | **                          |
| Stress period |              |                          |                   |                         |                           |                          |                             |
| Recomended planting date |          |                          |                   |                         |                           |                          |                             |
| Control       | 7.53g        | 77.5de                   | 3.547bc           | 226.583a                | 39.593g                   | 2.122bc                  |                             |
| 400           | 7.803fg      | 80de                     | 4.368b            | 214.417ab               | 40.063fg                  | 1.79a                    |                             |
| 800           | 9.42ef       | 82.5de                   | 4.723b            | 206.75bc                | 67bc                      | 1.788c                   |                             |
| 1200          | 11.232cd     | 165b                     | 4.918ab           | 187.75cde               | 68.253bc                  | 3.416bc                  |                             |
| 1600          | 10.655de     | 155b                     | 4.712b            | 172ef                   | 56.16de                   | 2.383bc                  |                             |
| 2000          | 9.082defg    | 102.5cde                 | 4.532b            | 178.333ef               | 48.77c                    | 3.142bc                  |                             |
| 2400          | 9.017defg    | 102.5cde                 | 4.252b            | 197.75bed               | 48.123ef                  | 2.283bc                  |                             |
| Late planting | Control      | 11.947bcd               | 140b              | 2.292c                  | 214ab                     | 61.9cde                  | 5.21bc                      |
|               | 400          | 13.053b                  | 143.5b            | 3.842b                  | 198.75bcd                 | 62.08cd                  | 2.267bc                     |
|               | 800          | 13.334b                  | 160b              | 4.342b                  | 185.25de                  | 76.623a                  | 2.716bc                     |
|               | 1200         | 15.607a                  | 205a              | 6.267a                  | 165f                      | 78.6a                    | 1.809c                      |
|               | 1600         | 12.728bcd                | 147.5b            | 4.855b                  | 170.25ef                  | 71.133ab                 | 3.468b                      |
|               | 2000         | 12.305bced               | 110c              | 3.745b                  | 184.167de                 | 63.663bcd                | 2.215bc                     |
|               | 2400         | 8.852fg                  | 67.5e             | 3.572bcd                | 208ab                     | 32.14fd                  | 2.479bc                     |

ANOVA

| date          | ***  | ***  | n.s.  | *    | ***  | *    |
| Priming       | ***  | ***  | **    | *    | ***  | **   |
| date × priming| ***  | ***  | *     | ***  | ***  | **   |

* Within each column, different letters indicate significant differences at P ≤ 0.05 (Duncan test). n.s., *, ** and *** indicate non-significant or significant differences at P, 0.05, 0.01 or 0.001, respectively.
This increment may be due to more reduction in $E$ than to $P_N$ in this phenological stage because of cool temperature in tillering stage. With some exceptions WUE was decreased by priming (Table 2, 3 and 4). Exceptions was found in late planting treatments in tillering and recommended planting in heading. It seems priming by SA enhanced water status of plants and RWC (Abdollahi and Shekari, 2013). It is reasonable from $E$ and $g_s$ data which increased in SA treatments compared to control treatments. Therefore, transpiration rate increased by SA application more than $P_N$ and WUE relatively decreased. Although, in our experiment WUE decreased by seed priming with SA, increasing in WUE by pretreatment with SA reported previously (Liu et al., 2011; Fariduddin et al., 2003; Khan et al., 2003).

**Chlorophyll Index and Chlorophyll Content**

In all growth stages first planting date had higher chlorophyll content index (CCI) compared to second planting date. Although, in first planting date heading stage had highest value among other phenological stages, but in late planting the highest amount of CCI was found in grain filling (Table 5). The lowest value among three growth stages was observed in tillering stage and second planted plants. In general, seed priming with 1200 $\mu$M of SA had greatest impact on increasing the amount of CCI in both planting date and in all three growth stages. The lowest values was observed control treatments and in late planting date.

Like CCI, chlorophyll a and b were affected by seed priming and planting date treatments (Table 5). The amounts of Chl a, b, total and a/b were increased through 1200 $\mu$M of SA treatment relative to control treatments in both planting dates. The amount of Chl b was increased to 10.5% through 2000 $\mu$M of SA compared to control treatment in late planting. Overall, the amounts of Chl a and b on the first planting date were higher than second planting date. However, priming through SA could discount the adverse effects of late planting and the amount of chlorophyll reduction. It seems that the effect of SA on biosynthesis and/or protection of chlorophyll a are more than chlorophyll b. Because the ratio of chlorophyll a/b in primed treatments in both planting dates are more than control treatments.

Chlorophyll content is important in maintenance of photosynthetic capacities (Jiang and Huang, 2001) and a key factor in determination of photosynthesis rate and dry matter production (dos Santos et al., 2013). Also, it is stated chlorophyll content is the most reliable parameter to estimate leaf growth and development (Albert et al. 2012). Gunes et al. (2007) reported in maize plants salt stress or application of SA had not significant effect on chlorophyll a, b and total content, but SA reduced carotenoids contentment. In contrast, Arfan et al. (2007) stated salinity decreased chlorophyll content of wheat, but SA increased chlorophyll content. Similarly, Sinha et al. (1993) pointed out that chlorophyll and carotenoid contents of maize were increased upon treatment with SA. Treatment with 500 $\mu$M SA for 24 h before exposure to chilling provided protection on Rubisco activity and chlorophyll content (Yordanova and Popova,
It seems that this effect of SA on photosynthetic pigments depends to types of species, cultivar, method of SA application and its concentrations.

**Total dry weight (TDW)**

In comparison of two planting date, first planting had more dry weight compared to second planting date. With some exceptions, in all growth stages in treatments which had higher photosynthetic rates, higher accumulation of dry matter was found (Table 2, 3, 4 and 5).

Table 5. Effect of priming by salicylic acid (SA) on, Chlorophyll content index (CCI) in three stages, chlorophyll (Chl) (a), (b), (a/b), (a+b) content, and Biological Yield (BY) at harvest.

| Planting date | Priming [µM] | CCI (Tillering) | CCI (Heading) | CCI (Grain filling) | Chl a [mg g⁻¹] | Chl b [mg g⁻¹] | Chl a+b [mg g⁻¹] | Chl a/b | BY [g m⁻²] |
|---------------|-------------|-----------------|---------------|---------------------|----------------|---------------|-----------------|---------|-----------|
| **Stress period** |             |                 |               |                     |                |               |                 |         |           |
| **Recommended planting date** | | | | | | | | | |
| Control        | 16.740abc  | 41.33cd         | 39.703def     | 0.8315f            | 0.3762abc     | 0.8132de      | 1.208ef        | 0.2936f | 2.273de   | 1237de   |
| 400            | 17.748bc   | 44.76ah         | 41.715cd      | 0.8543f            | 0.3913b       | 0.2936f       | 1.148ef        | 0.2936f | 2.910b    | 1437nc   |
| 800            | 22.4ab     | 45.533b         | 46.38b        | 0.9573ab           | 1.349b        | 0.4032b       | 1.564a         | 0.247cd | 1572ab    |
| 1200           | 28.210a    | 46.85a          | 48.95a        | 1.166              | 0.4032b       | 1.564a         | 2.875ab        | 1641a   |
| 1600           | 26.922a    | 46.313a         | 41.163ed      | 0.3843ab           | 1.469b        | 0.2843abc     | 1605a          |
| 2000           | 23.52ab    | 43.32bc         | 38.893ef      | 0.9434d            | 1.246de       | 0.3030ef      | 3.114ab        | 1549ab   |
| 2400           | 23.160ab   | 39.537de        | 36.07gh       | 0.986ed            | 0.331cdef     | 1.317cd       | 2.979g         | 1322ed   |
| **Late planting** | | | | | | | | | |
| Control        | 5.342f     | 28.53ef         | 33.923b       | 0.751fi            | 0.3372bcde    | 1.088gh       | 2.228de        | 969f     |
| 400            | 5.96de     | 36.173r         | 34.2h         | 0.7965fi           | 0.3106ef      | 1.107gh       | 2.575bcd       | 1237de   |
| 800            | 10.183d    | 37.903h         | 39.83de       | 0.7135ghi          | 0.3211ddef    | 1.035h        | 2.226de        | 1281d    |
| 1200           | 14.01cd    | 39.366e         | 43.105c       | 1.031bc            | 0.3234cdef    | 1.355         | 3.203c         | 1284d    |
| 1600           | 4.805r     | 37.354f         | 41.533cd      | 0.8415f            | 0.2841f       | 1.126g         | 2.963ab        | 1188de   |
| 2000           | 4.433r     | 32.333g         | 38.403ef      | 0.747ghi           | 0.3726bcd     | 1.120gh        | 2.008e         | 1105ef   |
| 2400           | 4.09r      | 31.46g          | 37.603g       | 0.628h             | 0.2911ef      | 0.919f         | 2.174de        | 1096ef   |

ANOVA

| date | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| Priming | n.s. | *** | *** | *** | *** | *** | *** | *** | *** |
| date × priming | ** | *** | *** | *** | *** | *** | *** | *** | n.s. |

* Within each column, different letters indicate significant differences at P \(\leq 0.05\) (Duncan test). n.s., *, ** and *** indicate non-significant or significant differences at P, 0.05, 0.01 or 0.001, respectively.
Increment in fresh and dry weight of plants by SA treatment may due to increase in cellular dividing rate in apical meristem of root and shoot of plants which enhance plant growth (Sakhabutdinova et al., 2003). Horvath et al. (2007) reported in wheat seedlings SA enhanced growth rate via increasing auxins and cytokinins concentrations. Also, Agami (2013) stated higher rate of dry matter production of maize plants both in normal and salt stress conditions by application of SA due to induction of antioxidant enzymes activities, proline and photosynthetic pigments. Increasing dry weight of artichoke plants by application of SA reported by Rajabi et al (2013) Fariduddin et al. (2003). On the other hand, Singh and Usha (2003) showed high concentrations of SA have preventive effects on wheat and maize growth. In our experiment seed priming with 2000 and 2004 μM SA concentrations had unfavorable effects on most recorded traits.

CONCLUSIONS

Presented results showed that change in planting date could affect photosynthetic parameters. In late planted plants PN was lower than conventional planting in tillering and heading stages, but in grain filling stage this trend was reversed. This trend approximately was found in gs and E traits. The highest values for PN, gs, E and CE were obtained in heading and then in tillering stages. In contrast, Ci was lower in heading stage and highest in grain filling stage. It seems that, in heading and flowering stage due to higher demand for photoassimilates PN showed higher values and in grain filling stage by aging of leaves capacity of photosynthesis relatively decreased. Seed priming by SA significantly increased PN and related parameters. Furthermore, the highest and lowest of WUE were achieved in tillering and heading, respectively. May be due to low temperature in early spring, the value of E was lower than other stages and this affect WUE in this stage. Seed priming with SA decreased WUE compared to control treatment in both planting dates. As shown by gs, it seems SA induced to more opening the stoma and therefore increment in E was more than PN. According to results, seed pretreatment with 1200 μM SA had appropriate performance than other SA concentrations.

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