# Do Phytohormones Influence the Grain Quality and Yield of Winter Wheat under Drought Conditions?

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***Abstract*—Two winter wheat (*Triticum aestivum* L.) cultivars (Sirvan & Pishtaz) were grown in greenhouse in College of Agriculture, Shiraz, Iran, under two water regimes (80%FC & 40% FC) after anthesis to evaluate the influence of two phytohormones (Salycilic acid and Strigolactone) on grain yield and quality. On grain samples, the following quality analyses were performed: grain hardness, protein and gluten content, gluten index and SDS test. Furthermore, Grain yield and thousand grain weight were measured as well. Results showed that protein content, grain hardness, wet gluten, gluten index and SDS were enhanced in drought condition. However, this rate dampened when pots sprayed with salycilic acid or strigolactone. A significant reduction in thousand grain weight and yield were observed in drought of both cultivars. This reduction was lower in cv. Sirvan. Exogenous application of salycilic acid and strigolactone diminish diverse effects of drought in plants. Restricted water during grain filling stage leads to lower yield but higher grain quality. Application of strigolactone and salycilic acid changed these results next to well watered situation.**

***Index Terms*—wheat, strigolactone, salycilic acid, drought**

## I. Introduction

Wheat (*Triticum aestivum* L) is one of the most principal crops all around the world and approximately one sixth of the total arable land of the world are dedicated to wheat production. Wheat is a key cereal crop for global food security as it is an important source of protein in more than 80 countries. Wheat and its derivatives provide necessary calories for about 30% of the world population [1]. It has been estimated that in the next forty years developing countries such as Iran will be responsible for 95% of the increased demand for cereal crops [2]. The most important responsibility of modern agriculture is to provide global food security in a sustainable way.

Wheat is predominantly milled to flour before being used as a food component. However, whole-wheat grains may be used to make foods as well. Bread, pasta, noodles, biscuits, and cakes are the most common foods made basically from wheat flour. Whole- wheat grains may be used as an ingredient to make foods such as breakfast cereals, curries, soups, and porridge. Grain of certain cultivars with good characters that make it suitable for bread making, rather than biscuit-making or livestock can often attract a price premium [3]. As we know, both protein content and protein quality can exert an influence on bread-making quality [4]. In many parts of the world, wheat is mainly use to make bread as the major staple food. The need for production of economic and nutritive foods in developing countries such as Iran has made wheat and other cereals an important source of raw material in food industry.

Wheat grain quality is a major concern in the research efforts worldwide. Differences Among wheat grain qualities are depend on the soil, nutrient supplies, biotic and abiotic stresses, standard of plant protection, and other agronomic factors [4]. Wheat is considered of to be the greatest influential among cereals because of its processing features. The main proteins stored at wheat endosperm are called gluten proteins which include gelyadin and glutenin. Environmental factors, such as temperature, water and nitrogen nutrition influence the ratio between soluble and insoluble proteins, affect bread-making quality, which is a function of the protein composition that is genetically controlled. Differences in grain quality in winter wheat cultivars were associated with changes in protein composition from drought stress occurs during grain filling. Evidently, timing is very significant in modifying the effect of stress on grain quality and yield formation [5].

Wheat grain quality cannot be simply defined since it changes depending on different conditions such as drought situations. Wheat is grown at irrigated as well as rain-fed conditions worldwide. At grain development stage, under rain-fed conditions the developing grains of wheat are exposed to mild or severe stress. Stress during the grain-filling stage may cause reduced grain-filling that has a great effect on wheat plants. It may also accelerated cell death, and an earlier attainment of the harvest maturity [6], which may leads to substantial changes in the protein composition and content and in the size distribution of starch granules of the wheat grains.

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Drought stress might also affect protein accumulation. For example Instead of the proteins that play an active role in the biosynthesis and metabolism, storage proteins and those involved in protecting the plants against biotic and abiotic stresses are accumulated preferentially. The protein content in flour increases significantly with bread wheat as the result of the drought stress [5]. Various types of stresses affect protein composition which is the most decisive factor in bread making quality [5]. It was demonstrated by Zhao *et al.* (2009) that drought stress during the later phenophases of grain-filling modify protein components [5]. In Iran, drought occur most frequently after anthesis, during the grain-filling period, which influences the quality of the wheat kernels.

The grain hardness is also one of the important distinguishing features of wheat plants for commercial purposes evaluating. Hardness and softness are the milling characteristics related to the way of the endosperm breaks down. The fragmentation of the kernels of hard wheats tends to occur along the lines of the cell boundaries. Some researchers believe that hardness is related to the degree of adhesion between starch and protein. Others think that hardness depends upon the protein matrix continuity [7].

The use of Plant Growth Regulators (PGRs) in agriculture to promote plant growth, production and quality is becoming increasingly more common. Environmental stresses such as drought which often cause complex physiological, molecular, and biochemical changes in plants, may alter the levels and ratio of different endogenous PGR. Under environmental stress conditions, it is reported that exogenous application of PGRs (seed priming or foliar application) may overcome much of the internal PGRs deficiency and could lead to reduced inhibitory effects caused by the stress [8]. From a practical point of view, application of PGRs offers a potential approach to mitigating the inhibitory effects of drought-stress on plant growth and crop productivity.

Salycilic Acid (SA) is a PGR that is part of signaling pathway induced by several biotic and abiotic stresses. SA is believed to play a major role in defense mechanism against drought stress [8]. Kaydan *et al.* (2007) reported that SA can play important roles in plant growth and development, photosynthesis-related processes such as stomatal regulation and ion uptake and transport. Eyshi and Bannayan (2012) reported that priming wheat seeds with salycilic acid can decrease protein content of seeds in drought condition as a consequence of longer grain filling time and higher starch to protein content [9].

Strigolactones (SL) constitute a new class of plant hormones which are active as germination stimulants for seeds of parasitic weeds of Striga, Orobanche , in hyphal branching of Arbuscular Mycorrhizal (AM) fungi and as inhibitors of shoot branching [10]. This phytohormones controls several different processes in plants [11]. By using different molecular and physiological approaches. Van Ha *et al.* provided credible evidence that, in Arabidopsis, SL acts as positive regulator of plant responses to drought and salt stress, which was associated with various factors such as shoot- rather than root-related traits [11].

Although the effect of drought stress on grain quality, yield and development of wheat is well documented [12], the studies on changes in quality of the mature grains of wheat, in response to phytohormones and water stress at post-anthesis are, however, scanty. It is, therefore, imperative that a comprehensive analysis of the influences of drought stress and phytohormones interaction on changes in the nutritional value of wheat grains be carried out. There is insufficient information on this subject in the literature. This paper seeks to further clarify phytohormones effects on grain quality of two cultivars of wheat using single different water conditions at post-anthesis stage.

## II. Material and Methods

### A. Plant Materials and Growth Conditions

This study was conducted at the greenhouse of the college of Agriculture, Shiraz University, Iran, during the 2015-2016 growing season using two wheat cultivars (Sirvan as a drought tolerant and Pishtaz as a drought sensitive). Minimum and maximum temperatures in the greenhouse were 14 and 28 ℃, respectively, and relative humidity was 55-60%. The wheat plants were grown to 14-h photoperiod. All seeds were surface- sterilized in 1% sodium hypochlorite for 10 min, rinse thoroughly with distilled water. Seeds of all two cultivars were sown in the plastic pots. The pots were filled with soil+sand in 2:1 ratio. After emergence the seedlings were thinned to five plants per pot. The soil fertilized by the application of urea at a rate of 150 kg $ha^{-1}$ (sowing time, mid tillering and anthesis stage). (Soil classification: fine, mixed, mesic Cacixerolilic Xerochrepts, and pH and electrical conductivity (EC) of the soil extract were 7.7 and 2.55 dS $m^{-1}$, respectively).

### B. Irrigation Regimes

Plants were watered to maintain at field capacity level before starting irrigation treatments (until anthesis stage). From anthesis to ripening drought treatment was applied to maintain 40% of field capacity, while control pots were irrigated to 80% of field capacity. Soil water content in each plot was measured using a TRIME-FM TDR (Time Domain Reflectometry, IMKO Micromodultechnik, Ettlingen, Germany). The percentage of maximum allowable depletion (MAD) of available soil water (ASW) in the root zone was estimated by Eq. [13].

$$\text{MAD}= \frac{\text{FC}-\theta}{\text{FC}-\text{PWP}} \quad (1)$$

where, FC is the soil volumetric moisture at field capacity and PWP is the soil volumetric moisture at permanent wilting point.

The volume of required water based on predefined MAD was calculated as (2) and (3):

$$\text{ASW}= \text{FC} - \text{PWP} \quad (2)$$

$$\text{Vd} = \text{MAD} \times \text{ASW} \times \text{Rz} \times 10 \quad (3)$$

where Vd is the volume of irrigation water (mm), and Rz is the effective rooting depth, and 10 is the conversion constant of cm to mm.

### C. Experimental Design and Treatments

The experiment was factorial based on Completely Randomized Design (CRD) with three replicates.

There were eight treatments for each wheat cultivar: no drought stress and no foliar application (wet, SA-, Sl-), no drought stress with Salycilic application (wet, SA+, SL-), no drought stress with Strigolactone application (wet, SA-, SL+), no drought stress with Salycilic acid and Strigolactone application (wet, SA+, SL+), drought stress without foliar application (dry, SA-, Sl-), drought stress with Salycilic application (dry, SA+, SL-), drought stress with Strigolactone application (dry, SA-, SL+) and drought stress with Salycilic acid and Strigolactone application (dry, SA+, SL+).

Salycilic acid (0 and 1 mM)in the form of sulphosalycilic acid dyhidrate which provided from Merck company and synthetic Srigolactones (SL) analogue GR24 (0 and 10 μM) that purchased from Professor B. Zwanenburg, University of Nijmegen, NL were applied with a hand sprayer until the solution began to drip off leaves at sunset. To assure SA and SL uptake by the leaves, they were applied on four consecutive days. The pots not receiving SA or SL were treated similarly with equivalent amount of distilled water.

### D. Chemical Analysis

Grain protein (%) content were determined by near-infrared spectroscopy calibrated based on official AACC methods 39–10 [14]. Wet gluten and gluten index (The gluten index is the ratio of the wet gluten remaining on the sieve (after centrifugation) to the total wet gluten) was calculated by the (AACC) Approved Method 38-12.02. For measuring SDS sedimentation testing (Sedimentation volumes (values) reflect differences in both protein quantity and protein quality) the approved AACC 56-70 method was followed [15].

### E. Statistical Analysis

The total data were subjected to the analysis of variance (ANOVA) using statistical program SAS 9.1 and the means were seperated by LSD test ($P \leq 0.05$).

## III. Results and Discussion

### A. Thousand Grain Weight and Crop Yield

Thousand grain weight decreased significantly ($P \leq 0.05$) in both cultivars when plants were subjected to water stress (40% FC). In cereals starch biosynthesis happens by using simple carbohydrates in grain filling stage [16]. Decline in the rate of grain growth resulted from reduced sucrose synthase activity, while cessation of growth resulted from inactivation of adenosine diphosphate-glucose- pyrophosphorylase in the water stressed wheat [17]. Thousand grain weight in drought- tolerant (Sirvan) wheat cultivar was higher than that in the relatively drought-sensitive (Pishtaz) cultivar under water stress conditions (Fig. 1). However exogenous application of Strigolactone (GR24) significantly improved 1000 grain weight under stress conditions (Fig. 1-A). A reduction in grain filling occurs due to a reduction in the assimilate partitioning and activities of sucrose and starch synthesis enzymes [18].

Foliar application of Salycilic Acid at anthesis stage accelerated thousand grain weight of wheat plants significantly under water stress situation in comparison to plants without Salycilic acid application (Fig. 1-B). This effect was more pronounced in pots which Salycilic acid and Strigolictone were used together (Fig. 1-C). Khodary (2004) reported that foliar application of Salycilic acid improved water use efficiency of maize crops [19]. Exogenous application of Salycilic acid dramatically reduced the transpiration rate in common bean [20]. Such reduction in transpiration may be beneficial in reducing water loss under drought stress condition. Recently some papers published about the role of Strigolactones in abiotic stress situations although with some contradictions [11]. Our results show that plant that received GR24 withstand negative effects of drought conditions much better. Ha *et al.* (2014) argue that strigolactones crosstalk with Absicic acid (ABA) is a key point for higher drought tolerance in plants which received artificial strigolactones [11]. Stomatal closure induced by ABA is promoted for maintaining water status in plant cells under water-deficit conditions [21]. In the study on the effects of exogenous GR24 in drought condition in model plant Arabidopsis indicated that SL acts as a positive regulator of stress signaling networks and diverse ABA signaling pathways by regulating the expression of many stress and/or ABA-responsive genes which has a role in plant development and abiotic stress response.

Crop yield (g per plant) of wheat cultivars significantly affected by drought stress. However this reduction was smaller in drought tolerant cultivar (Fig. 2-A). Yield reduction in pots with Salycilic application was much lower in drought condition. (Fig. 2-B). Applying strigolactone on plants enhanced their yield in 40% of field capacity. (Fig. 2-C). One thousand grain weight has been reported as a promising characteristic for increasing crop yield in wheat in various conditions. AS we discussed applying exogenous strigolactone and salycilic acid leads to higher thousand grain weight as well as more crop yield. Many scientist reported wheat yield loss as a result of reduction in the rate and duration of grain filling in drought [22].

### B. Grain Protein and Hardness Index Percent

Grain protein content (%) significantly affected by drought stress. Protein content in both cultivars increased when encountered drought stress at grain filling stage. (Fig. 2-D) By spraying Salycilic acid, protein content of grains declined significantly. (Fig. 2-E) Lower grain protein content resulted from foliar application of GR24 on plants (Fig. 2-F). Our results are in agreement with Guzman *et al.* 2015 who found that wheat grain protein were inversely correlated with thousand grain weight [23]. Since drought reduce green leaf area and the plants efficiency to fix dry matter after anthesis and during the

grain filling period, less starch is accumulated in the Grain, and resulted in higher final grain protein concentration [24]. Salycilic acid application improve several characteristics such as stability of membranes, chlorophyll a and b content and regulation of key phytosynthetic reactions such as activity of RUBP carboxylase in plant that reduce adverse effects of drought stress [25]. These effects adjust the ratio among protein and starch content of grain and decline the protein content of seeds with salycilic acid application. As we mentioned previously, strigolactones are efficient in drought situations since they regulate absicic acid rate which can recovery the stomatal closure in plants [11]. In this regard plants that sprayed by strigolactones in drought conditions can survive the situation better than other plants. As a result they have higher grain weight and lower protein content.

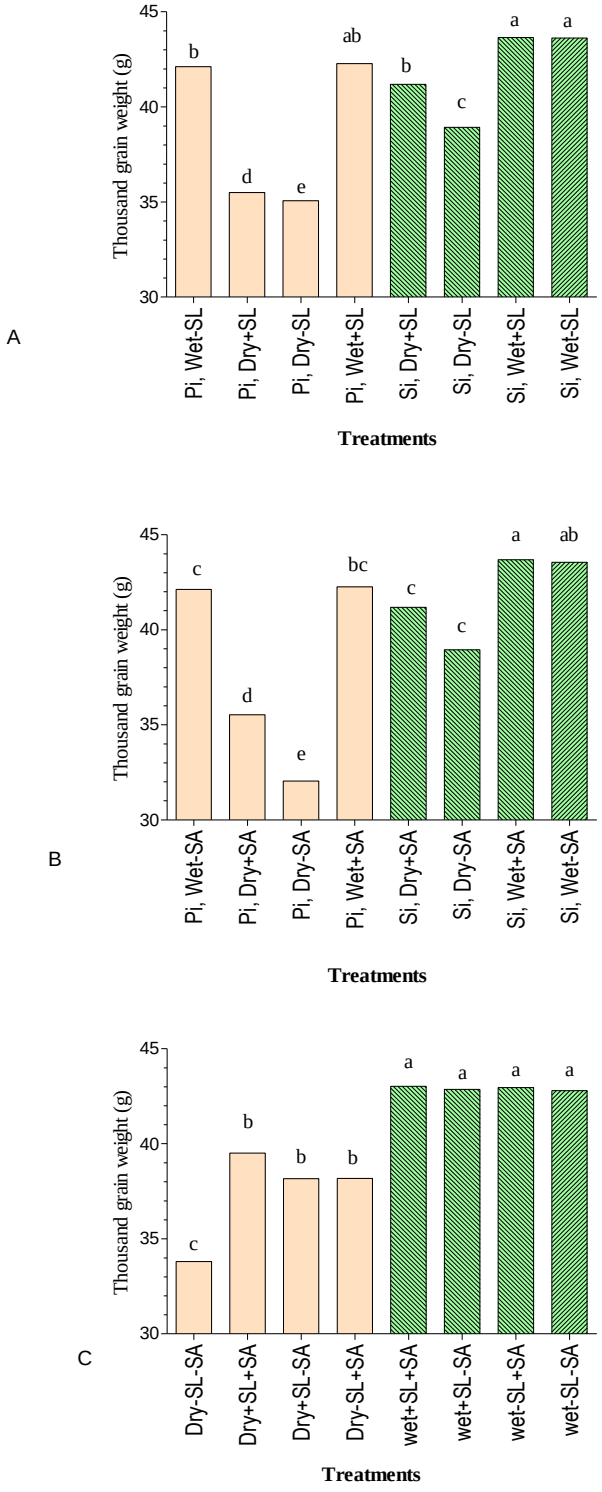


Figure 1. Effects of irrigation regimes, salycilic acid and GR24 application on thousand grain weight (g) of two wheat cultivars Pi (pishtaz, drought sensitive) and Si (sirvan, drought tolerant). In each figure columns with the same letter are not significantly different (LSD test, P≤0.05)

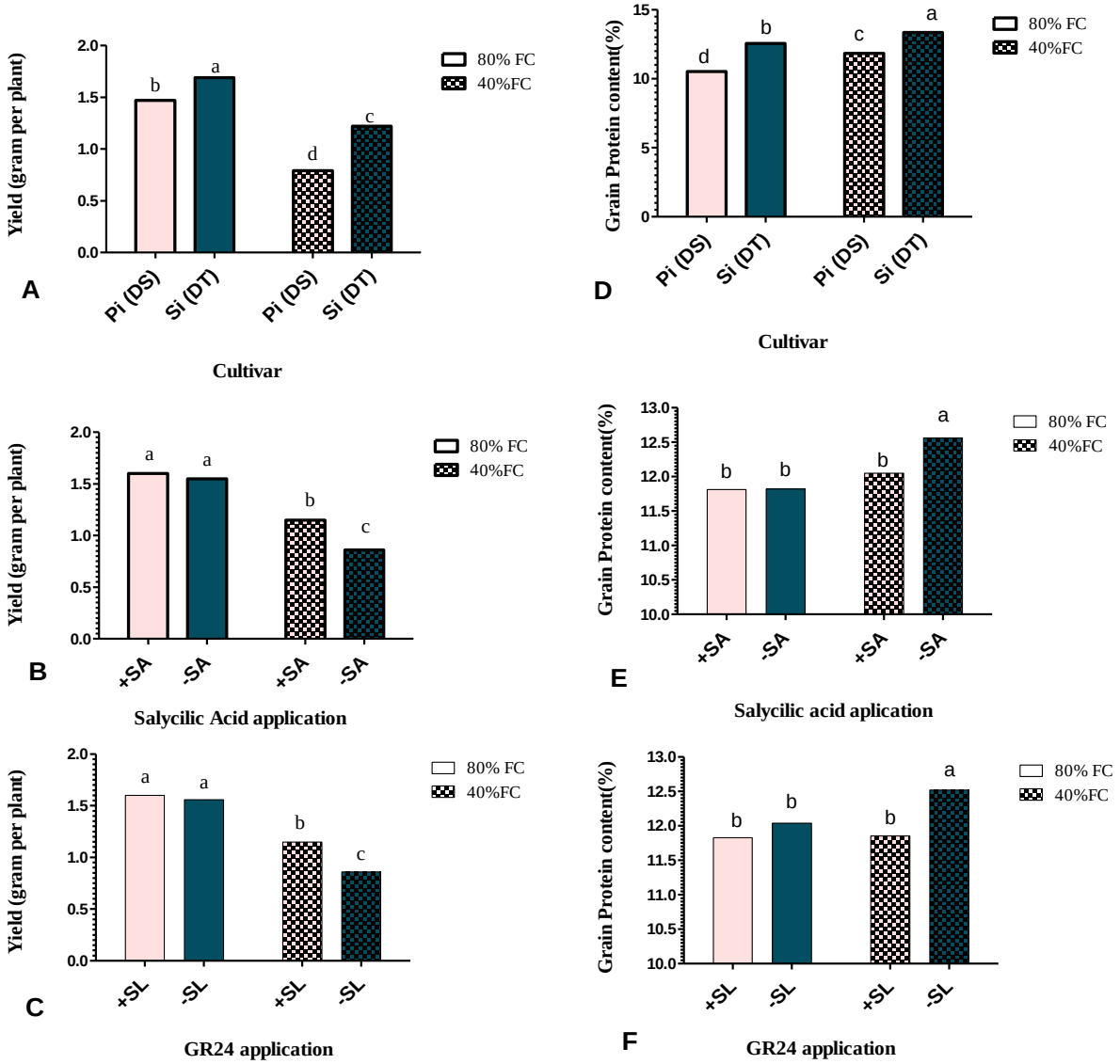


Figure 2. Effects of irrigation regimes, salycilic acid and GR24 application on crop yield (g per plant) and protein content of two wheat cultivars Pi (pishtaz, drought sensitive) and Si (sirvan, drought tolerant). In each figure columns with the same letter are not significantly different (LSD test, P≤0.05)

TABLE I. EFFECTS OF IRRIGATION REGIMES, SALYCILIC ACID AND GR24 APPLICATION ON HARDNESS INDEX AND SDS OF TWO WHEAT CULTIVARS PISHTAZ (DROUGHT SENSITIVE) AND SIRVAN (DROUGHT TOLERANT). IN EACH FIGURE COLUMNS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT (LSD TEST, P≤0.05)

| Cultivar | Water stress | Salysilic acid and GR24 foliar application | SDS | Hardness index (%) |
|---|---|---|---|---|
| Sirvan (Drought tolerant) | 80% FC | -SL-SA | $50.86^{a}$ | $45.60^{f}$ |
| | | -SL+SA | $50.88^{a}$ | $45.81^{f}$ |
| | | +SL-SA | $50.81^{a}$ | $45.85^{f}$ |
| | | +SL+SA | $50.85^{a}$ | $45.89^{f}$ |
| | 60% FC | -SL-SA | $42.71^{e}$ | $48.06^{c}$ |
| | | -SL+SA | $45.44^{c}$ | $47.54^{d}$ |
| | | +SL-SA | $45.22^{c}$ | $47.40^{d}$ |
| | | +SL+SA | $48.36^{b}$ | $47.47^{d}$ |
| Pishtaz (Drought sensitive) | 80% FC | -SL-SA | $43.16^{d}$ | $46.38^{e}$ |
| | | -SL+SA | $43.98^{d}$ | $46.54^{e}$ |
| | | +SL-SA | $43.92^{d}$ | $46.44^{e}$ |
| | | +SL+SA | $44.26^{d}$ | $46.27^{e}$ |
| | 60%FC | -SL-SA | $40.34^{g}$ | $49.82^{a}$ |
| | | -SL+SA | $41.77^{f}$ | $48.45^{b}$ |
| | | +SL-SA | $41.68^{f}$ | $48.53^{b}$ |
| | | +SL+SA | $42.03^{f}$ | $47.47^{d}$ |

Means with the same letters in each coumn are not significantly different (LSD test, P≤0.05)

Analyze of variance table showed that application of Salycilic acid and GR24 in drought condition on grain hardness for wheat cultivars was statically meaningful ($p \leq 0.05$) (Table I) Hardness index was highest in cv. Pishtaz in drought condition without receiving Salycilic acid or GR24. Our result showed that there is a direct relation between protein content and grain hardness which is in consistent with the conclusion of researchers that discovered more protein content leads to higher hardness index [24]. This supports the findings of Glenn and Johnston (1994) and Dobraszczyk (1994) who found that vitreous grains were harder [26]. Since both protein content and vitreosity increased under drought, it would be predictable that the two characters were related. However, it is not accepted by some researchers [27]. The other theory is that drought increased hardness through a change in grain structure or density associated with reducing grain size [24]. In this regard, every factor that can increase or decrease grain protein (drought and phytohormones respectively), might have the same effect on hardness index. Wheat having high protein content tends to be hard, have strong gluten, and produce good qualitybread.

### *C. Wet Gluten, Gluten Index and Sds*

The results proved that drought situation increased wet gluten in both cultivars significantly. However, this reduction was much lower in drought tolerant cultivar (Sirvan) (Fig. 3-A). This is most likely due to association among protein content and wet gluten. Ozturk and Aidin (2004) reported higher wet gluten in drought situation in wheat [28].

Salycilic acid application on plants in drought situation reduced wet gluten content of grain (6.69%) (Fig. 3- B). Wet gluten was decreased significantly by spraying GR24 on plants in drought situation. (8.40%) (Fig. 3-C). These results are probably due to effects of strigolactone and salycilic acid positive effects in drought situation. Their effects increased grain weights and as result higher protein content and wet gluten.

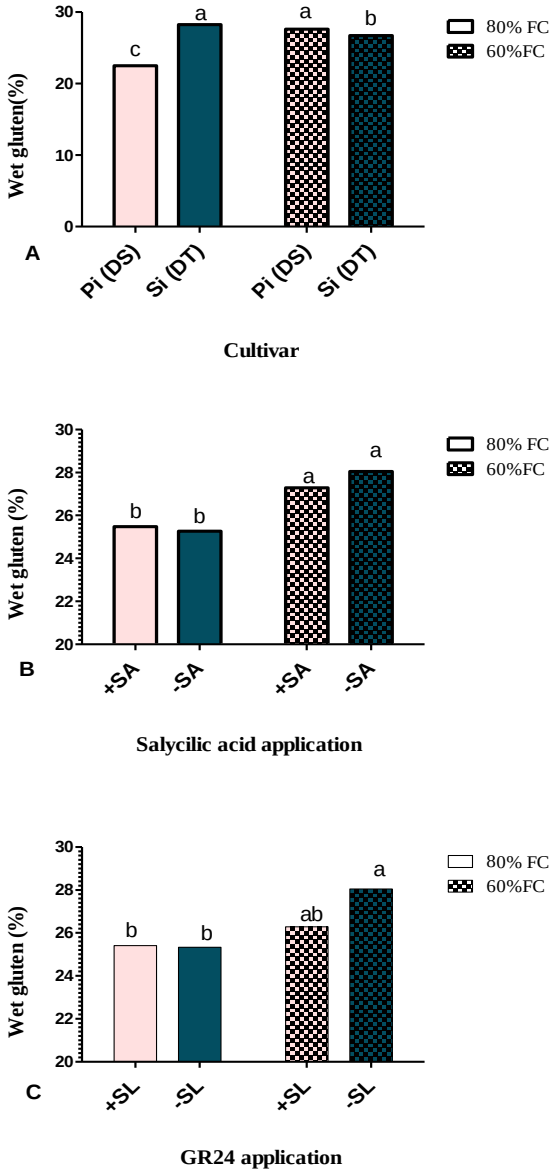


Figure 3. Effects of irrigation regimes, salycilic acid and GR24 application on wet gluten content of two wheat cultivars Pi (pishtaz, drought sensitive) and Si (sirvan, drought tolerant). In each figure columns with the same letter are not significantly different (LSD test, $P \leq 0.05$)

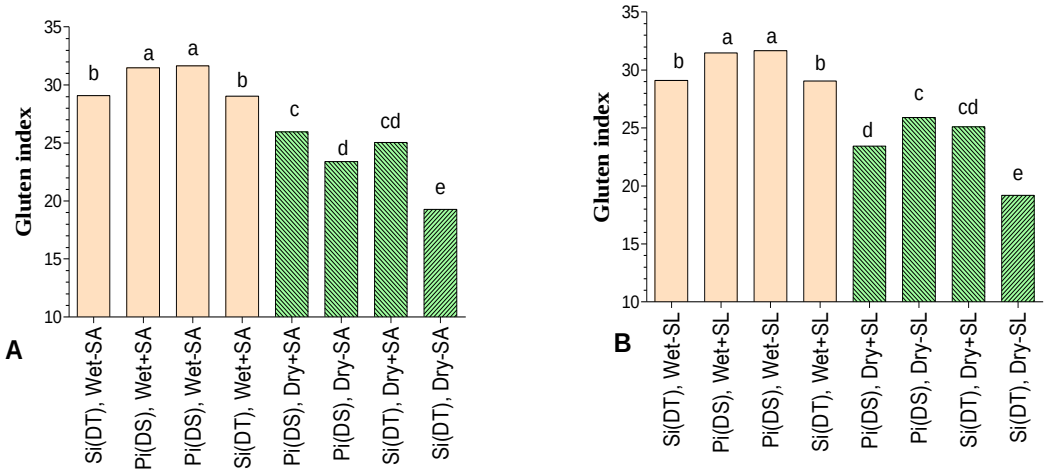


Figure 4. Effects of irrigation regimes, salycilic acid and GR24 application on gluten index of two wheat cultivars Pi (pishtaz, drought sensitive) and Si (sirvan, drought tolerant). In each figure columns with the same letter are not significantly different (LSD test, P≤0.05)

Gluten index was lower in water restricted condition in both cultivars significantly. Using Salycilic acid in 80% field capacity was not meaningful in each cultivar. However, in drought situation application of salycilic acid enhanced gluten index rate. (Fig. 4-A). GR24 had the positive effect on gluten index of wheat grains in drought situation in drought sensitive and drought tolerant cultivars. (Fig. 4-B) Wheat of low protein content tends to be soft, have weak gluten, and produce small loaves of inferior crumb structure, but produce better quality cookies. To produce bread with better quality from Iranian wheat cultivars, the protein content should be 11-13% [29].

From analyze of variance it could be resulted that salycilic acid, strigolactone application and wheat varieties in drought situation had meaningful effect on SDS (p≤0.05) (Table I). The highest SDS observed in drought tolerant cultivar (Sirvan) without drought stress. There was a significant difference among two cultivars. In pots that received exogenous salycilic acid and GR24, SDS rate increased meaningfully. In drought tolerant cultivar (Sirvan) this amplification was accelerated when phytohormones used together in comparison to use each one alone. (Table I). Significant increases in sedimentation volume due to water stress after anthesis were related to differences in protein content. Sedimentation values reflect quantity of protein and rate of dough development [30].

## IV. Conclusion

In this study two wheat cultivars revealed differences in response to drought and exogenous application of salycilic acid and strigolactones. In contrast to grain yield, grain quality was favored by drought. The results presented here, show the positive role of strigolactone and salyilic acid in drought situation. However, this role is not completely identified. Further research is required to find the way that plants passed to overcome pernicious drought effects when phytohormones added to their shoot.

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