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Role of Plant Growth Regulators in Gene Expression of SGR Gene Responsible for Stay Green of Wheat Varieties

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ABSTRACT

A field experiment was carried out during winter season of 2019-2020 at Al-Mhanawyah Research Station - Agriculture Research Directorate - Babylon Governorate / Iraqi, to study the gene expression of Sgr gene responsible for controlling the duration of staying green in varieties of wheat under effect of plant growth regulator during the two growth stages (vegetative and reproductive) by using quantitative reverse transcription-PCR (RT-qPCR) technique and achieving the highest grain yield for a number of wheat varieties. Randomized complete block design (RCBD) arranged according to split plots used with three replicates. The experiment included twelve wheat varieties (Saberbic, Al-Rasheed, Iraq, Tamoz-3, Al-Adnaniya, Babel, IPA-99, Al-Latifeya, Al-Hashemia, Al-Naeema, Buhooth-10 and Al-Baraka) within main plots and nine treatments of plant growth regulator spraying (spraying of distilled water at tillering and anthesis stages, spraying of Abscisic acid (ABA) at tillering stage, spraying of ABA at anthesis stage, spraying of ABA acid at tillering and anthesis stages, spraying of kinetin at tillering stage, spraying of kinetin at anthesis stage, spraying of kinetin at tillering and anthesis stages, spraying of ABA at tillering stage + kinetin at anthesis stage and spraying of ABA at anthesis stage + spraying of kinetin at tillering stage) within sub plots. The results showed that ABA spraying caused a significant increase in the gene expression of Sgr gene, whereas the spraying of kinetin caused a significant decrease in gene expression. It was observed that the Saberbic variety gave the highest gene expression of Sgr gene (1.44). Al-Rasheed and Iraq varieties showed that the decrease in the vegetative growth period which accounted 65% of lives cycle compared with reproductive stage (35%). Also, It is observed that the spraying of ABA was decreased the number of days of the stage in which it is sprayed or after, while the spraying of kinetin was increased the number of days of the stage in which it is sprayed or after. Besides, the spraying of kinetin at tillering stage revealed a significant increase in the biological yield (11.68-ton ha^{-1}), whereas the spraying of ABA at tillering stage + kinetin at anthesis stage had the highest mean of grain yield of (4.63-ton ha^{-1}). Al-Rasheed variety gave the highest mean of grain yield (5.56-ton ha^{-1}).

Keywords: Sgr gene, Plant growth regulators, kinetin, Abscisic acid, Wheat, RT-qPCR, Gene expression, Chlorophyll, Stay green.

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INTRODUCTION

Iraq is one of the original places for growing wheat in which all the necessary elements for its production are available. However, its production is below the required level for several reasons, the most important of which is the failure to adopt modern management practices in serving of the field in the critical stages of its life: the vegetative and the reproductive growth (Hobbs and Morris,1996).

The appropriate use of plant growth regulators is one of these practices that helps to face the problems of growth and development of the crop and raise the yield, as they play a stimulus role in increasing yield, (Srivastava et al., 2016). According to Rouphael and Colla (2018) plant growth regulators are chemical and agricultural tools to increase the efficiency of the crop by using nutrients to get benefit of its best physiological and genetic capacities. Hence, plant growth regulators are modified of growth rather than being nutrients. Moreover, they work to understand the processes of generation of the yield components through their influence on plant growth and development (Fahad et al., 2016). Arising from these facts, paying more interest to growth stages results in increasing the wheat yield. In other words, knowing plant's critical growth and its requirements of growth regulators to deal with growth and development problems will achieve the best results (Rouphael and Colla, 2018). Certain genes are responsible of the vegetative and reproductive growth duration, and as it is called the staying green trait, which is also called "genetic late leaf aging". The gene Sgr is the most important one among them; it is a recessive gene on the 9th chromosome that deletes the yellowing of the plant (Thomas and Ougham, 2014). It contributes to the gene expression of the building protein chlorophyll A and B. However, increasing its expression causes rapid aging because it promotes the degradation of chlorophyll through reducing the number of thylakoids in grana by ccE enzymes (Rosidah and Hilhorst, 2017). Hence, it can also be called a Sgr gene, with a non-yellowing gene (NYE_1) encoding magnesium dechelatase, which stimulates the conversion of chlorophyll A to pheophytin A. Staying green trait affects on grain yield by retaining chlorophyll content, improving the ability of photosynthesize and increasing the grain filling period. Several studies have highlighted the possibility of affecting the gene expression of staying green genes through using plant growth regulators (Wang and Chekanova, 2016). Growth regulators can be added to regulate plant growth and limit vegetative growth during the reproductive phase, as the continuation of vegetative growth during this phase causes increased competition for nutrients and reducing the yield (Jung and Rademacher, 2017). Furthermore, other studies have indicated that the use of plant growth regulators during different stages of growth, especially critical ones such as tillering and anthesis, contributes to stimulating physiological and biochemical processes, as they participate in building proteins and carbohydrates,

and are also believed to be responsible for cell division and the production of some natural hormones by which the yield increases (Hashim and Ahmed, 2017). Kinetin contributes to delaying the aging of the leaves as it had an effect on the synthesis of the IAA-Oxidase enzyme. Also, ABA (abscisic acid) activates the process of defoliation of the leaves, as it works to accelerate the dissolution of the middle plate and components of the cell wall and works to speed synthesis of pectase, cellulose and protease enzymes that accelerate the leaf shedding process (Yuen et al., 2020). The effectiveness of plant growth regulators may be change due to the change of environmental conditions, time of spraying, as well as the different of genetic structures of wheat, so genetic diversity and the adoption of different and high-productivity varieties are among the necessary strategies for food security through knowing the behaviors and performance of each variety and its response to growth factors. Accordingly, the spraying of growth stimulators or impediments at appropriate times coincides with the stages of formation and growth of the components of the grain yield (emergence and growth of tillers and spikes, the emergence and growth of spikelets and florets and filling the sites of the grain) will increase these components and then the grain yield (Leprince et al., 2017). Consequently, the present research aims to study the gene expression of Sgr gene responsible for controlling the duration of staying green in varieties of wheat under effect of plant growth regulator treatments during the two growth stages (vegetative and reproductive) by using quantitative reverse transcription-PCR (RT-qPCR) technique and achieving the highest grain yield for a number of wheat varieties.

MATERIALS AND METHODS

A field experiment was carried out during winter season of 2019-2020 at Al-Mhanawyah Research Station - Agriculture Research Directorate - Babylon Governorate / Iraqi, to study the gene expression of Sgr gene responsible for controlling the duration of staying green in varieties of wheat under effect of plant growth regulator during the two growth stages (vegetative and reproductive) by using quantitative reverse transcription-PCR (RT-qPCR) technique and achieving the highest grain yield for a number of wheat varieties. Randomized complete block design (RCBD) arranged according to split plots used with three replicates. The experiment included twelve wheat varieties which were: Saberbic (V1), Al-Rasheed (V2), Iraq (V3), Tamoz-3 (V4), Adnaniya (V5), Babel (V6), IPA-99 (V7), Al-Latifeya (V8), Al-Hashemia (V9), Al-Naeema (V10), Buhooth-10 (V11) and Al-Baraka (V12), within main plots and nine treatments of plant growth regulator spraying which were: spraying of distilled water at tillering and anthesis stages (S0), spraying of Abscisic acid (ABA) at tillering stage (S1), spraying of ABA at anthesis stage (S2), spraying of ABA at tillering and anthesis stages (S3), spraying of kinetin at tillering stage (S4), spraying of kinetin at anthesis stage (S5), spraying of kinetin at tillering and anthesis stages (S6), spraying of ABA at tillering stage + kinetin at anthesis stage (S7) and spraying of ABA at anthesis stage + spraying of kinetin at tillering stage) within sub plots (S8). Kinetin's concentration used was 100 mg L^{-1}, and the concentration of ABA was 16 mg L^{-1}. The targeted growth stages in the present study were determined according to the scale of Zadoks et al. (1974). Soil management especially plowing was carried out as required, and then the experiment land was divided into 324 experimental units, the area of each experimental unit was (3 m long x 3 m width) 9 m^2 which contained 15 lines, 0.20 m apart. The seed of wheat varieties were sown on the 15 Nov. 2019 at a rate 120 Kg ha ^{1}. Recommended nitrogen fertilizer was added as a urea (46% N) with an average 200 kg N ha^{-1} at four doses (1/4 at planting, 1/4 at ZGs13, 1/4 at ZGs32 and 1/4 at ZGs40) according to wheat growth stages (Jadoo, 1995), while the phosphorus fertilizer was added as a super triphosphate (46% P_2O_5) with an average 100 kg P_2O_5 ha^{-1} before the planting (Farhood, 2014). Crop management were carried out as needed, and the plants were harvested after the appearance of maturity signs.

RNA Extraction

Ten samples (leaf) were taken from each experimental unit at the vegetative stage (10% tillering stage) and at the reproductive stage (10% anthesis). Then the RNA was extracted by using the ZR Plant RNA kit. MiniPrep™, produced by the US company Zymo, and then the manual of the company that supplied the special RNA extraction kit (Kat. No R2024) was followed.

RT-qPCR to measuring Sgr Gene Expression

The RT-qPCR test of the ribonucleic acid was performed for the study treatments using the GoTaq® qPCR Master Mix (Cat.No A6120) kit supplied by Promega, then placed in the real time PCR and the program was carried out as shown in Table (1).

Table 1. Real Time PCR Reaction Conditions Program

The Step	Temperature (Cº)	Time	Cycle Number
Reverse transcription	43.0 °C	10 min	Hold
Enzyme activation	96.0 °C	3 min	Hold
Denaturation	96.0 °C	15 sec	40
Annealing/Extension	59.0 °C	15 sec	

After the interaction was completed, the data were analyzed and Livak and Schmittgen's (2001) method was used to estimate the relative gene expression of the Sgr gene through the following equations:

$$\Delta_{ct} = ct_{badh2} - ct_{GAPDH}$$

$$\Delta\Delta_{ct.control} = \Delta_{ct.test} - \Delta_{ct.control}$$

$$\textbf{Gene Expression} = 2^{-\Delta\Delta ct}$$

As:

ct_{badh2}: is the cycle threshold for a target gene (Sgr). ct_{GAPDH}: is the cycle threshold for the reference gene (GAPDH).

$\Delta_{ct.test}$: is the difference between the cycle threshold of the target gene and the reference gene for the samples tested?

$\Delta_{ct.control}$: is the difference between the cycle threshold for the target gene and the reference gene for the control samples.

Studied Traits

Measurements were taken for a number of field traits as shown below:

1. Number of vegetative growth days (day): It includes the number of days from germination to the emergence of spikes.
2. Number of reproductive growth days (day): It includes the number of days from the emergence of spikes until harvest.
3. Chlorophyll index (SPAD): The chlorophyll index of the flag leaf was calculated by using the SPAD device by taking the average of readings of 10 flag leaf for the main stems randomly from each experimental at eight stages (seedling, tillering, stem elongation, booting, emergence of spikes, anthesis, milk phase stage, dough phase stage and ripening phase stage (Pask et al., 2012).
4. Grain yield (ton ha^{-1}): Square meter was harvested from each experimental unit, dried and then the grain weight was weighted and converted from g m^2 to ton ha^{-1}.
5. Biological yield (ton ha^{-1}): Square meter was harvested from each experimental unit, dried and then weighed completely (stems + leaves + spike) and converted from g m^2 to ton ha^{-1}.

RESULTS AND DISCUSSION

The results in the Figure (1) reveal that the spraying treatments were differed at the cycle threshold values (CT) of the SGR gene at different growth stages. The spraying od ABA at tillering stage (S1) caused a significant decrease in the CT values of the Sgr gene at the vegetative stage and gave 23.93 cycles, this result was non- significant difference with the spraying of ABA at tillering + anthesis stages (S3) and spraying ABA at tillering stage + kinetin at anthesis stage (S7), this lack of moral difference results from the fact that the CT calculation was done at the branching stage, before applying the second part of the treatment (spraying of ABA at anthesis stage and spraying of kinetin at anthesis stage), thus these two treatments are completely similar to the S1 treatment when calculating CT at the vegetative stage. The decrease of CT value is an indicator of an increase in gene expression, and vice versa. It is also signified that the spraying of kinetin at tillering stage (S4) caused a significant increase in the CT value at the vegetative stage and gave 28.23. This is an indication of a decrease in the value of gene expression. Hence, it also did non- significant difference with the spraying of kinetin at tillering + anthesis stages (S6) and spraying of ABA at anthesis stage + kinetin at tillering stage (S8), this lack of moral difference results from the fact that the CT calculation was done at the branching stage, before applying the second part of the treatment (spraying of kinetin at anthesis stage and spraying of ABA at anthesis stage). Therefore, these two treatments are completely similar to treatment (S4) when calculating CT at the vegetative stage. It was noticed, when calculating the CT values at the reproductive stage, that there was non-significant effect of spraying ABA at tillering stage (S1) and the spraying of kinetin at the tillering stage (S4). This fact denoted a tentative finding that the influence of these regulators are temporary due to the spraying was done at tillering stage. Also, the spraying of kinetin at anthesis stage (S5), spraying of kinetin at tillering + anthesis stages (S6) and spraying of ABA at tillering stage + kinetin at anthesis stage (S7) caused a significant increase in CT values at the reproductive stage and gave 33.39, 33.99 and 33.26 respectively, while the spraying of ABA at anthesis stage (S2) led to a significant decrease in the CT value at the reproductive stage (26.18) with non-significant difference with the spraying of ABA at tillering + anthesis stages (S3) and spraying of ABA at anthesis stage + kinetin at tillering stage (S8).

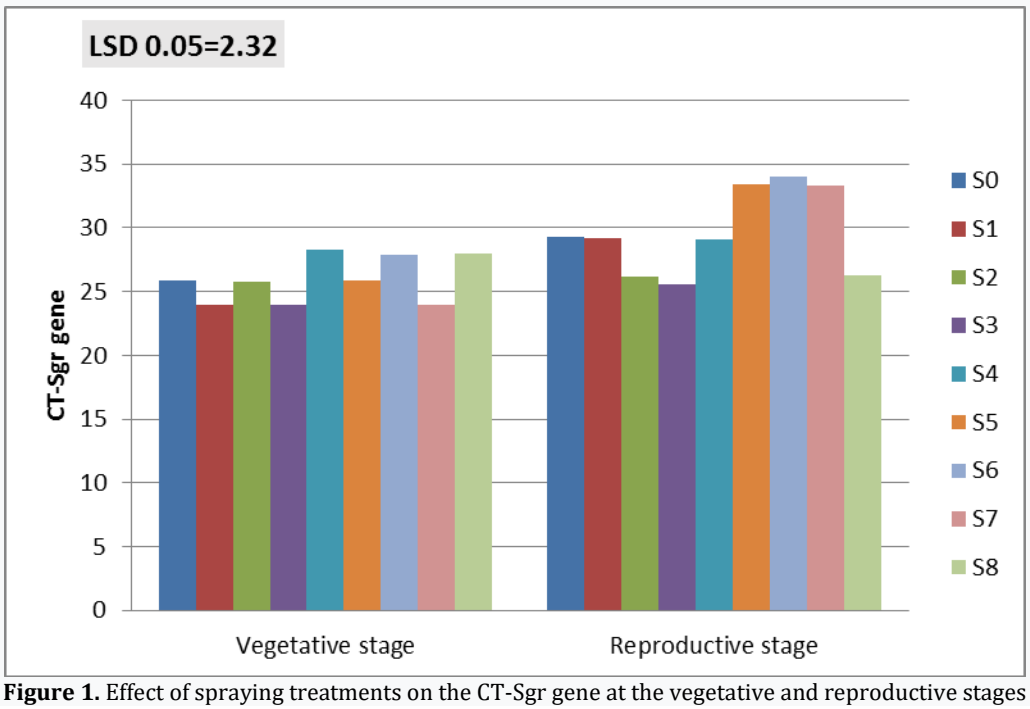

Figure 1. Effect of spraying treatments on the CT-Sgr gene at the vegetative and reproductive stages

The results in the Figure (2) indicate that there were significant differences among wheat varieties in the CT of Sgr gene values. Saberbic (V1) and Al-Rasheed (V2) varieties had the highest means of CT values at vegetative stage (28.8 and 27.08) respectively, whereas Buhooth-10 (V11) and Al-Baraka (V12) varieties had the lowest

means of CT values at vegetative stage (23.01,23.04) respectively. Moreover, Al-Rasheed variety (V2) was recorded highest mean of CT values at reproductive stage (32.13), while Al-Hashemia variety (V9) recorded the lowest mean of CT values in reproductive stage (26.77).

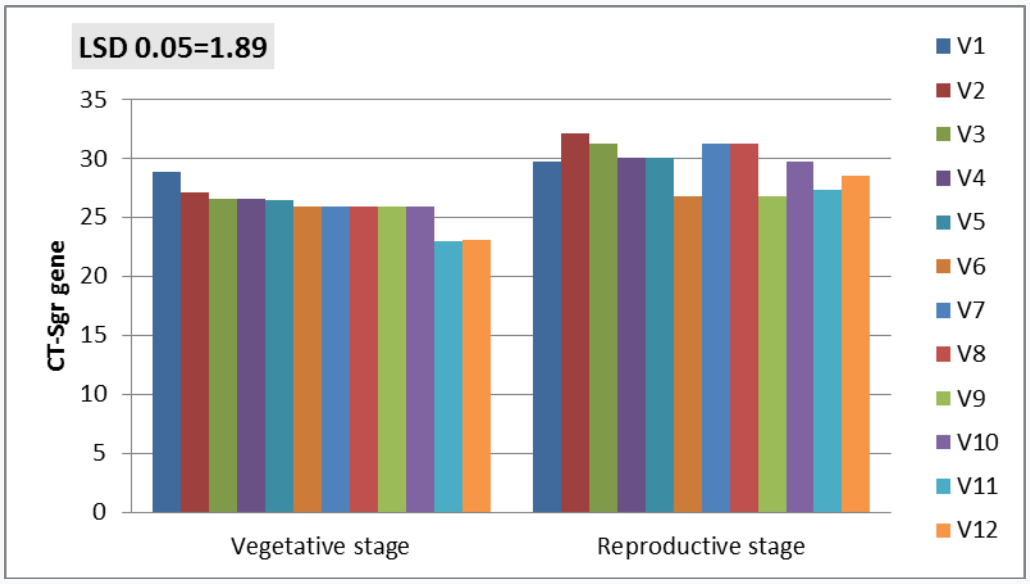

Figure 2. Effect of wheat varieties on the CT-Sgr gene at the vegetative and reproductive stages

The results in the Figure (3) show that there were significant differences between spraying treatments at the growth stages on the gene expression values, the spraying ABA at tillering stage (S1) caused a significant increase in gene expression at the vegetative stage (2.41 times) with non-significant difference with the spray of ABA at tillering + anthesis stages (S3) and spraying of ABA at tillering stage + kinetin at anthesis stage (S7), while the spraying of kinetin at tillering stage (S4) caused a significant decrease in gene expression at the vegetative stage (0.60 times) with non-significant difference with the spraying of kinetin at the tillering + anthesis stages (S6) and spraying of kinetin at tillering stage + ABA at anthesis stage (S8). Also, there was non-significant difference between sparying of kinetin and ABA at anthesis stage due to the gene expression was calculated at the vegetative stage as these treatments were not applied yet.

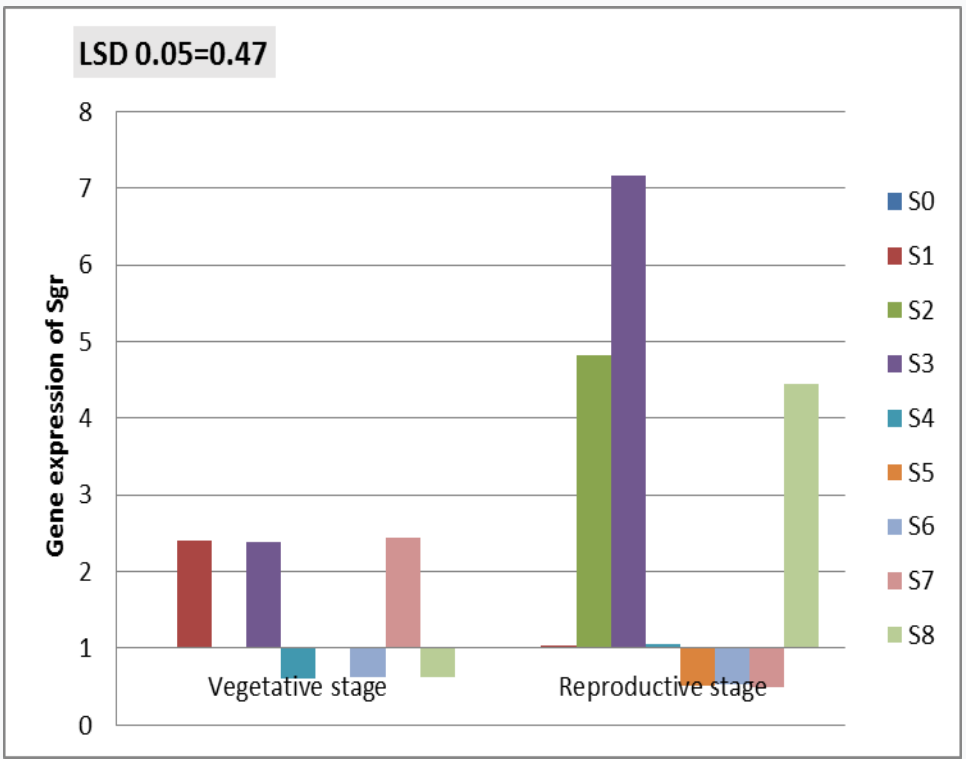

Figure 3. Effect of spraying treatment on the gene expression of Sgr gene at vegetative and reproductive stages

However, it was noted that the spray of ABA at tillering + anthesis stages (S3) caused an increase in gene expression of Sgr gene at the reproductive stage (7.17 times), whereas the spraying of ABA at tillering + the

spray of kinetin at anthesis stage (S7) caused a decrease in the gene expression of Sgr gene at the reproductive stage (0.50 times) compared to control treatment (S0). Such difference in gene expression values may be due to use of growth regulators that may participate in forming complex constructions to regulate the gene expression of stay green (Sgr) gene (Latif et al., 2020), or attributed to the effect of growth regulators on transcription factors, as these factors play a fundamental role in increasing or decreasing the gene expression (Jiang et al., 2007). Omidbakhshfard et al, .(2015) referred that the growth regulators play role in increasing or decreasing the gene expression through affecting on the gene transcription factors of the plant including: histone acetyltransferase activity, iron ion binding, transcription cofactor activity, oxidoreductase activity, protein binding and ion channel activity which could be caused increasing or decreasing the gene expression of Sgr gene.

It was noticed from Figure (4) that there were significant differences between wheat varieties during the vegetative and reproductive stages on the gene expression of Sgr gene, Saberbeck variety (V1) gave the highest mean of gene expression of Sgr gene (1.44 times), while Al-Baraka variety (V12) gave the lowest (1.26 times) during the vegetative stage, while a high gene expression was observed during the reproductive stages in general for the varieties compared to the vegetative stage. Al-Rasheed variety (V2) recorded the highest mean (2.69 times) during the reproductive stage, while Babel (V6) and Al-Hashemia (V9) varieties gave the lowest means (2 and 2 times) respectively. The difference in SGR gene expression between the varieties may be due to the difference in the sequence of the Sgr gene that will led to the difference in the gene expression of Sgr due to the occurrence of potential mutations (Latif et al, 2020).

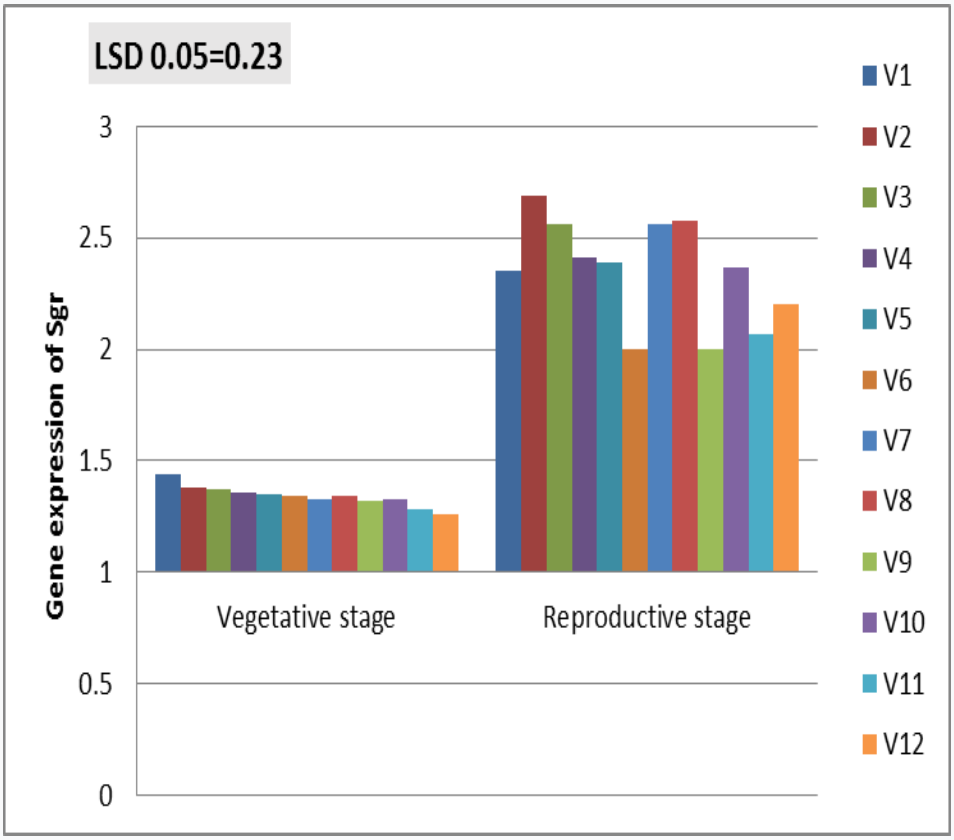

Figure 4. Effect of wheat varieties on the gene expression of Sgr gene at the vegetative and reproductive stages

Figure (5) shows the effect of spraying treatments on the life period of wheat. the spraying of ABA reduced the number of days of the stage in or after it is sprayed, while the spraying of kinetin increased the number of days of the stage in or after it is sprayed. The spraying kinetin at tillering stage + ABA at anthesis stage (S8) gave a vegetative growth period 68.76% of the life of wheat plant, while the spraying of ABA at tillering stage + kinetin at anthesis stage (S7) gave a vegetative growth period that accounted for 64.39% of the life of wheat plant, while the reproductive period accounted for 35.61%, compared to the control treatment (S0) which gave a vegetative growth 66.97% and reproductive growth 33.03% of the life of wheat plant. Such variation is attributed to the role of growth regulators (ABA and kinetin) in regulating the gene expression of SGR gene, as ABA which increased the gene expression (Fig. 3) and an increasing the chlorophyll destruction and then shortens the growth period, while the kinetin which reduced gene expression, hence, prolongs the growth period as a result of preserving chlorophyll at high levels.

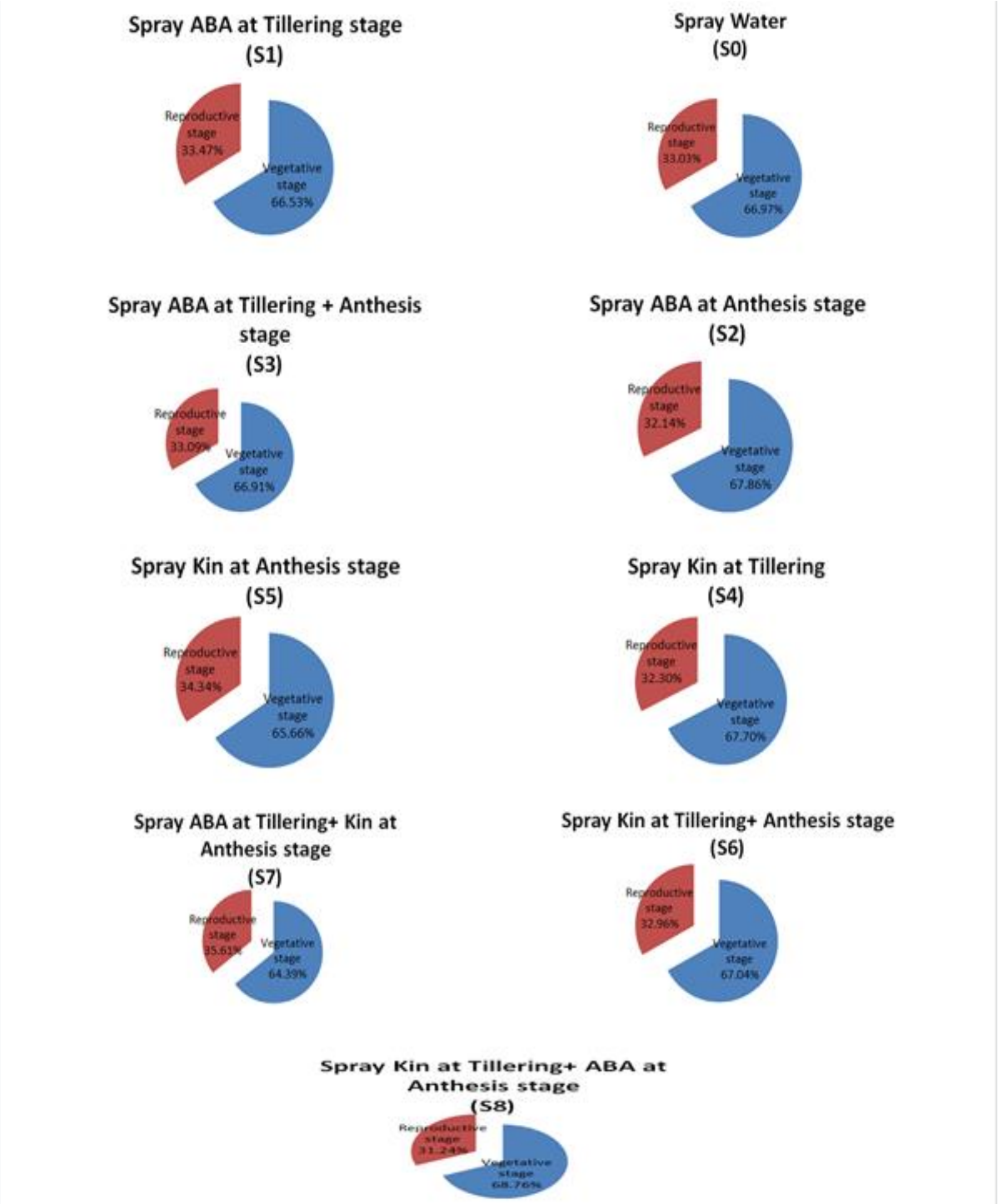

Figure 5. The effect of spraying treatments on wheat growth period

The results in the Figure (6) exposed that there are significant differences among the varieties in the period of vegetative and reproductive growth of the life cycle of the wheat plant, Al-Rasheed (V2) and Iraq (V3) varieties had the shortness of the vegetative growth period, which constituted 65% of its life cycle compared with the period of reproductive growth that reached 35%, while Al-Hashemia variety (V9) had a long period of vegetative growth, which constituted 70% of its life cycle compared with the period of reproductive growth that reached 30%. The difference in the period of vegetative and reproductive growth among the varieties may be attributed to their difference in the gene expression of Sgr gene. The high gene expression of Sgr gene will shorten the growth period, while the low gene expression will prolong the growth period. It has been observed that some varieties have low gene expression during the vegetative growth period as opposed to the reproductive growth period which will cause a deterioration in the grains yield due to the short period of the grain filling.

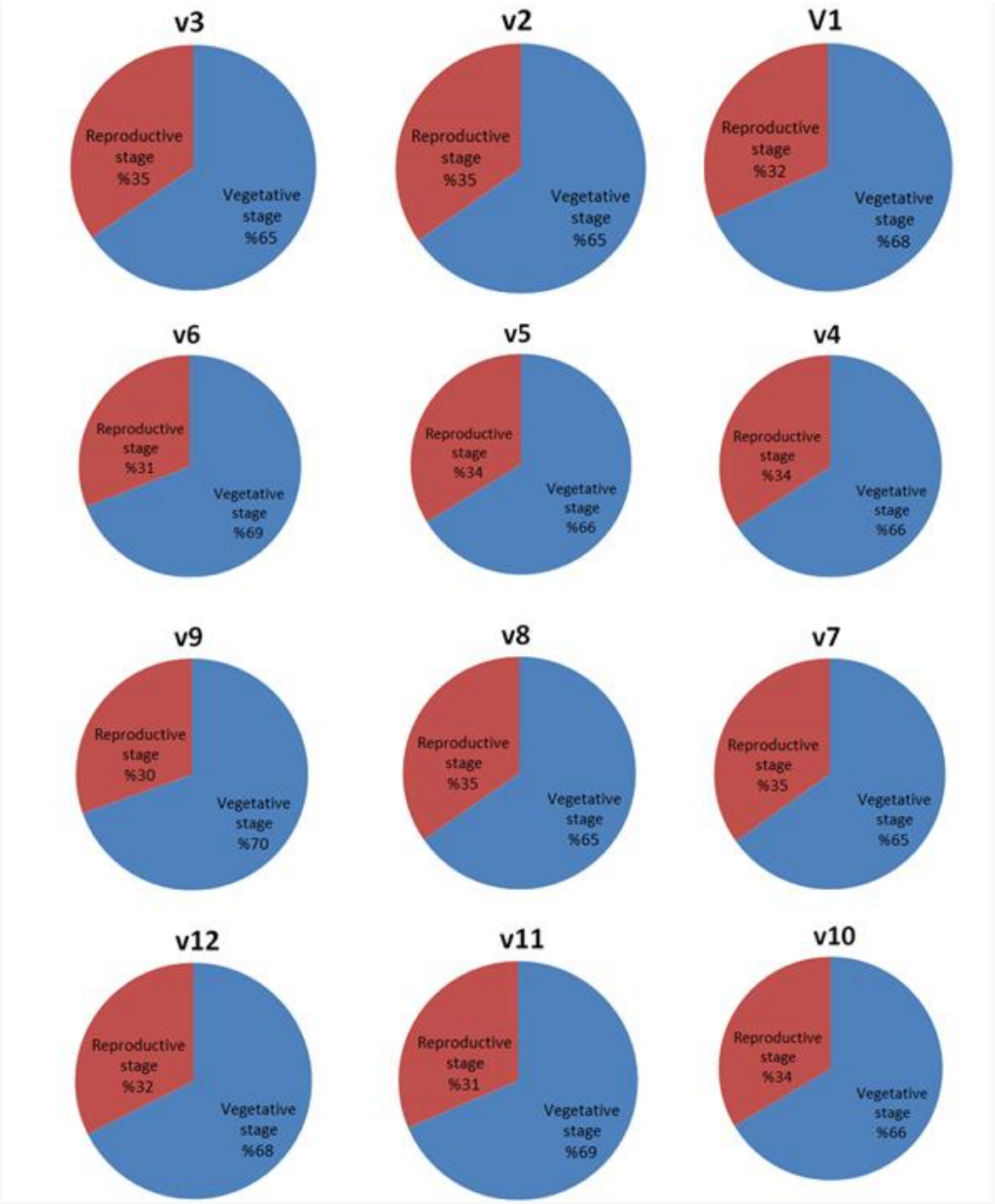

Figure 6. Effect of wheat varieties on the growth period

The results in the Figure (7) show that there were significant differences among the spraying treatments in the chlorophyll content during the growth stages. The study exposed that the chlorophyll begins to rise gradually, starting from tillering stage (44.58 SPAD) up to its highest value at the booting stage (49.86 SPAD) and then begins to decline after 20 days of anthesis stage down to its minimum at the maturity stage (13.57 SPAD). Generally, at any stage of plant growth, the spraying of ABA caused a significant decrease in the chlorophyll, while the spraying of kinetin caused a significant increase in the content of chlorophyll, and their effect begins to diminish gradually. The chlorophyll value for spraying of ABA at tillering stage (S1) was 44.66 SPAD with non-significant difference with the control treatment (S0) due to the measurement was done before the spraying, and then the effect of the treatment becomes evident when the plant reached the stem elongation stage, as the treatment caused a significant decrease in chlorophyll (7.92%). This decrease continued throughout the elongation and booting stages (7.00% and 6.98%) respectively, and then it decreases gradually from that of the control treatment until it becomes 1.37% at the emergence of spikes stage, and then its effect began to decline at other stages. However, the chlorophyll value when spraying the kinetin at tillering stage (S4) reached 43.67 SPAD, and then it began to increase at the stem elongation by a rate of 3.43% compared with control treatment, and this increase continued through the booting stage (3.52%), and then this increase began to

decrease when spikes emergence stage which reached 0.30%, with non-significant difference with the control treatment (S0), and then the effect of kinetin disappeared through the remaining stages. Also, the results show that the spraying of ABA at anthesis stage (S2) led to a significant decrease in the chlorophyll reaches 13.85% and this decline from the control treatment (S0) holds on during the milk and dough stages, and its effect disappeared at the maturity stage which non-significant difference with the control treatment (S0). Also, the spraying of kinetin at anthesis stage (S5) caused significant increased in the chlorophyll (4.57%), and this promotion from the control treatment holds on during the milk and dough stages, and then it faded at the maturity stage. Accordingly, it is highlighted that the effect of the growth regulator holds on for some stages and then gradually disappears. Hence, the reduction or increase in the number of days are resulted from the effect of growth regulators during these affected stages only. These results are consistent with Majeed et al., (2011) who indicated that the spraying of ABA led to reduce the vegetative growth stage and accelerate anthesis stage as result to role of ABA in urging the plant to accelerate anthesis and transit early from the vegetative growth stage to the reproductive stage.

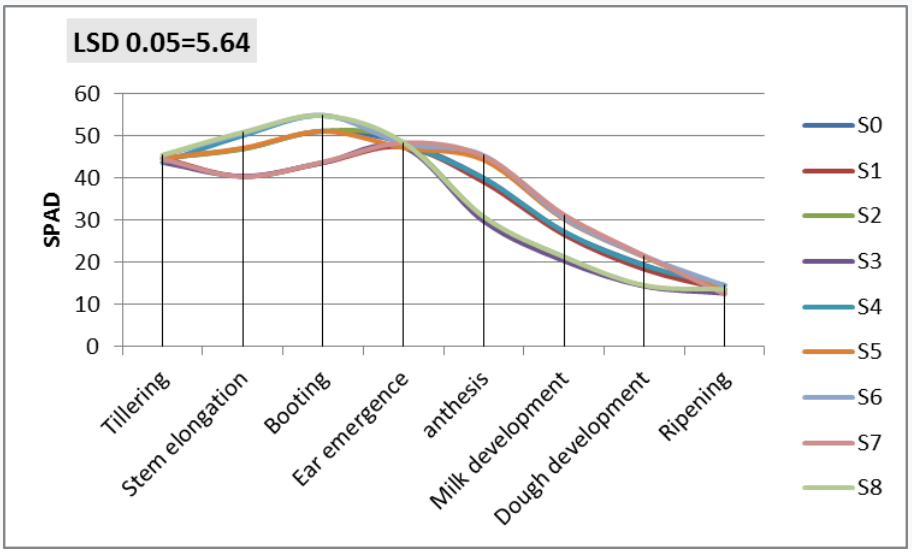

Figure 7. Effect of spraying treatments on the chlorophyll content (SPAD) during growth stages

The results in the Figure (8) indicate that there was significant difference among wheat varieties in the chlorophyll values during growth stages. Saberbic variety (V1) recorded the highest mean of chlorophyll content at booting stage, then it began to decrease till the maturity stage, while Buhooth-10 variety (V11) recorded the lowest mean (48.19 SPAD). In general, during the growth stage, Tamoz-3 variety (V4) recorded the highest mean of chlorophyll content (37.59 SPAD), while Buhooth-10 variety (V11) recorded the lowest (33.93 SPAD). The differences among varieties could be due to their genetical components and in their ability to form and retain chlorophyll (Al-tamimi, 2019).

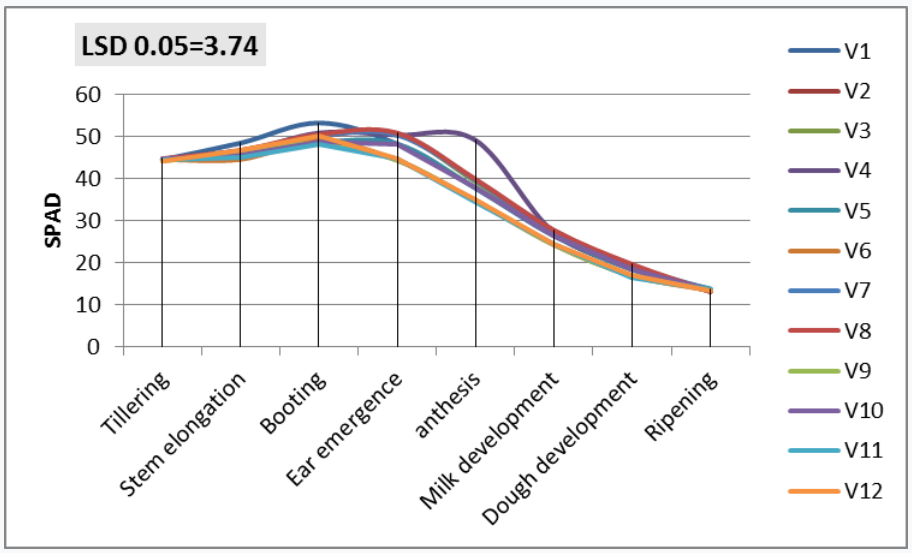

Figure 8. Effect of wheat varieties on the chlorophyll content (SPAD) during growth stages

The results in the Figure (9) reveal that there were significant differences among spraying treatments in the biological. The spraying of kinetin at tillering stage (S4) caused a significant increase in the biological yield (11.68-ton ha^{-1}) compared with spraying of ABA at tillering + kinetin spraying at anthesis (S7) which gave

the lowest mean of biological yield (8.92-ton ha^{-1}). These results may be due to the role of kinetin in an increasing the duration of vegetative growth (Fig. 5) which allowing more time to form a large vegetative system, in addition to, this increase may be caused by the spraying of growth regulators at early stages of wheat crop growth especially at the tillering stage leads to positive results in an increasing the dry matter and its distribution to the harvested parts of plant. These results are in agreement with Hussein (2015) who reported that the spraying of kinetin at tillering stage of wheat led to significant increase in the biological yield. Regarding of grain yield, the result in the Figure (9) indicates the spraying of ABA at tillering stage + kinetin at anthesis stage (S7) was significantly superior and gave the highest mean of grain yield (4.63-ton ha^{-1}) compared with spraying of ABA at anthesis stage + kinetin at tillering stage (S8) which gave the lowest mean (2.39-ton ha^{-1}). The superior of S7 treatment could be due to the role of ABA in decreasing the vegetative growth period and the role of kinetin in increasing the reproductive growth period (Fig. 5) which allowed the largest period of time for dry matter accumulation and grain filling.

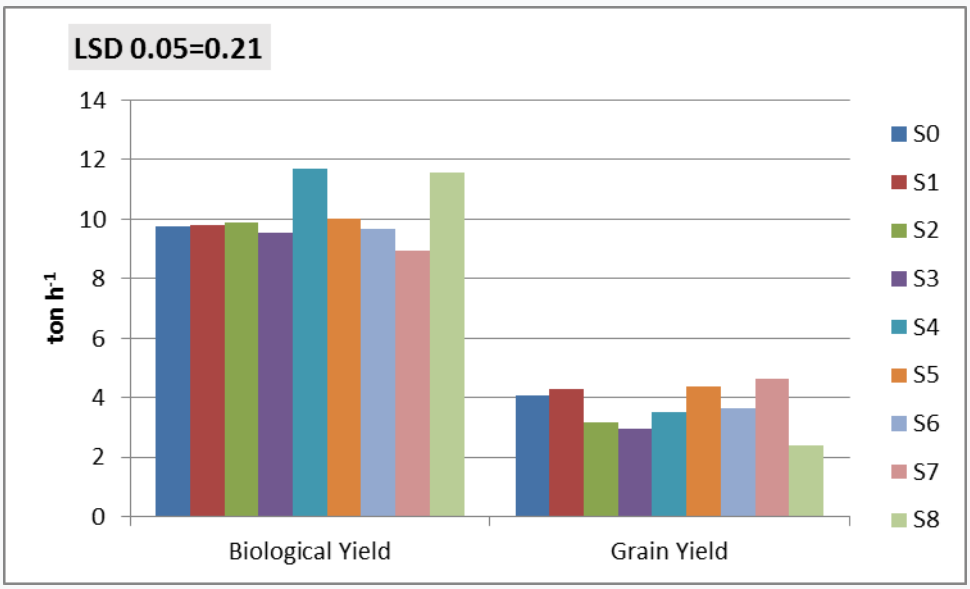

Figure 9. Effect of spraying treatments on the biological and grain yield (ton ha ^{1}) of wheat

The results in the Figure (10) reveal that there were significant differences among wheat varieties in the biological and grain yield. Saberbic variety (V1) gave the highest mean of biological yield (11.86-ton ha^{-1}) while Buhooth-10 variety (V11) gave the lowest mean (9.17-ton ha^{-1}). Regarding the grain yield, Al-Rasheed variety (V2) exceed other varieties by giving the highest mean of grain yield (5.56-ton ha^{-1}) whereas Buhooth-10 variety (V11) gave the lowest mean of grain yield (2.70-ton ha^{-1}). The reason of these differences among wheat varieties may be due to the difference in their ability to form the yield components (Al-tamimi, 2019), and their divergence in the periods of vegetative and reproductive growth (Fig. 6).

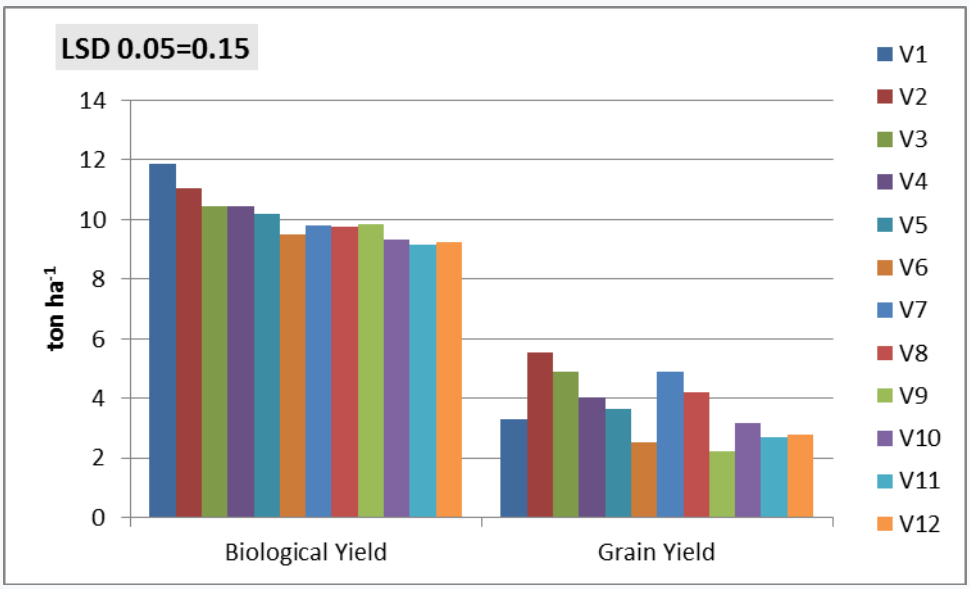

Figure 10. Effect of wheat varieties on the biological and grain yield (ton ha ^{1})

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