

ARTICLE / INVESTIGACIÓN

Phenotypic and genotypic investigation of three weeds residues allelopathic effect on the growth of three hybrid wheat cultivars

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Abstract: The present study was carried out to determine the allelopathic effect of extraction of three herbs (*Silybum marianum* L. and *Malva parviflora* L. and *Lolium rigidum* L.) on three wheat cultivars (Rashid, Abo Ghrib and IPA99) on germination, growth and ear formation by classical research methods (laboratory and greenhouse experiments and molecular detection of GA3-oxase2 (TaGA3ox2-1) gene expression and its crucial role in wheat growth and Ta14S gene expression as a gene responsible for ear development. The study analyzed the influence of weed residues on the germination and growth of three wheat cultivars. According to the mean effect of aqueous weed extract on the cultivars, the laboratory experiments revealed a significant difference in all the characteristics studied.

Key words: Weeds, hybrid wheat cultivars, allelopathy, RT-PCR.

Introduction

Wheat (*Triticum aestivum*) is a grass that belongs to the Poaceae family and is a widely cultivated agronomic crop for grains used in household consumption and the creation of a multitude of processed materials, which was labeled as a profitable agricultural venture; the crop has a long and illustrious history in human culture and civilization. It is essential to the world economy and food security¹. Weeds can produce many allelochemicals; they have a range of effects on other plants, including reducing or activating the germination and growth of receiving plants². Allelopathy from specific weed species impacts crop development; allelochemicals from allelopathic weed products can harm budding crop seedlings by disrupting root and shoot growth³. Allelochemicals secreted from *Chenopodium murale* L. root hairs were found to be responsible for cell cycle disruption and oxidative damage in wheat⁴. Allelochemicals are a type of secondary metabolite that isn't necessary for metabolism; they have been linked to decreased seed germination and seedling growth; their inhibition is complicated, involving interactions between various chemical classes such as phenolic chemicals, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids³.

Allelopathic water extracts from weed species, such as *Malva parviflora* L., were also found to limit barley growth and photosynthetic activity⁵. While found that adding aqueous extract vegetative sections of *Malva rotundifolia* L., *Silybum marianum*, and *Sonchus oleracens* to wheat seedlings reduced plumule and extreme length and weight⁶. Allelopathic weeds have been reported in enormous numbers; they harm crop plants from emergence through maturity, causing significant economic losses⁷. A varied range of weeds in wheat-growing fields is one of the critical limiting factors in wheat output⁸. Compared to wheat infested with weeds (*A. fatua* and *S. Marianum*), the results showed that wheat grown in weed-free circumstances had the highest biomass

and leaf area index during both growing seasons. To reduce crop-weed conflict and increase crop output, control of certain weeds, namely *A. fatua* and *S. marianum*, is particularly desirable⁹. Many kinds of research were achieved overall the world. In a seed bioassay, (10) utilized aqueous extracts of different extracts of four common weeds (*A. fatua*, *Phalaris minor*, *Melilotus alba*, and *Chenopodium album*) and found allelopathic effects on wheat at levels of 6,8,10, and 12g/100ml. The allelopathic effect of weeds (*Avena fatua*, *Melilotus officinalis*, and *Polypogon hissaricus*) on germination, growth, dry biomass and chlorophyll concentration of three wheat cultivars was discovered by (11).

Gibberellins (GAs) are plant hormones that control many growths and developmental processes in plants¹². They are especially critical in stem elongation regulation^{13,14}. As a result, it's reasonable to look for a link between GAs and plant height heterosis. In previous research, GA levels have been linked to the robust plant development seen in hybrid F1 plants. There are three lines of evidence that support this relationship. In maize, hybrids have greater GA levels than parental inbreds¹⁵, while an interspecific hybrid between *Liriodendron chinense* and *L. tulipifera* has higher GA levels than parental inbreds¹⁵. There are three phases in the production of GA in higher plants. As a molecular biomarker, we chose the GA20ox gene, which is involved in the third stage of GA biosynthesis and is linked to a critical role in the corrosion precursor of bioactive GAs to GA1. The bioactive GA is a factor in to increase in hybrid shoot cylinders than in inbred shoot cylinders¹⁶. Many experiments, on the other hand, focus on molecular mechanisms of developmental regulation that have the potential to accelerate wheat improvement. Even though 14-3-3 proteins are increasingly being linked to developmental control¹⁵. In the formation of wheat seeds, little is known about such genes. Because hexaploid There are three types of wheat chromosomes. We focused

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on the relevance of the Ta14S gene in wheat cultivars under study to better understand a regulatory function in wheat seed development¹⁷. In many scientific domains, molecular research utilizing quantitative polymerase chain reaction (qPCR) is regarded as a gold standard technology for confirming traditional results. The activation of relevant genes for numerous metabolic pathways is detected by screening at the molecular level of allelopathic action, which leads to increased plant germination, growth, seedling, flower, and fruit formation¹⁸.

One of the most severe issues in agricultural productivity is weeds. They have an impact on crops through direct resource competition as well as allelopathic consequences. Allelochemicals can be found in almost every part of a plant, including the leaves, flowers, fruits, roots, rhizomes, seeds, and pollen.

Materials and methods

In 2021, the research was carried out at Mosul University's Biology Department College of Science. Three weeds (*Silybum marianum* L., *Lolium rigidum* L., and *Malva sylvestris* L.) were collected and transported to the laboratory for allelopathic testing after harvesting. Wheat seeds were obtained from the sources testing and confirmation center in Ninawa governorate/Iraq.

Laboratory assay

Preparation of aqueous extracts

Weeds were mixed and stored for 24 hours at room temperature in distilled water (5gm per 100ml). After that, the solutions were filtered twice: once through a double layer of muslin gauze and then again through Whatman No.1 filter paper. These water extracts include 5% (w/v) water. The effect of water extracts at concentrations 5 % (w/v) was evaluated in a Petri dish bioassay. Ten wheat cultivars (Rashid, Abu Graib and IPA99) seeds were placed in sterilized Petri dishes lined with twice-folded filter paper. In each Petri dish, 8 ml of (5%, w/v) of three weed extracts was added, while distilled water was used in control. There were three frequencies in all of the treated samples. The dishes were incubated at 22 °C(±2) for 7 days in a Completely Randomized (CR) Design. The percent of seeds germination was calculated in the following equation¹⁹.

$$\text{Germination percent} = \frac{\text{Number of germination seeds}}{\text{Number of cultivative seeds}} \times 100$$

Seedling (cm), plumule and radical length (cm), in addition to plumule and radical fresh weight (mg), were measured at the end of this study.

Preparation of weed residues

In 2021, a pot experiment was carried out at the Department of Biology's greenhouse. The effect of incubated weed residues (5%, w/w) during one week in pots with soil was determined, 10 seeds were placed in Plastics pots with a diameter of 20cm for each of the wheat cultivars (Rashid, Abo Ghrib, and IPA-99) at 0.5cm depth from the surface of the soil, then irrigated with water and placed in a greenhouse at a temperature of 22 (±2)°C, 10 wheat seeds were sowed in the soil without residues as a control. After 60 days of seedling, the germination percent was assessed using a complete randomized block design, with each pot having three frequencies²⁰.

Phenotypic characteristics investigation

Germination percent, shoot length(cm), fresh weight of shoot(gm), spike length, number of leaves per plant, spike weight(g), and weight of 100 seeds. The water content (WC) can be expressed on a dry weight (DW) or fresh weight (FW) basis, as listed in the following equation²¹.

$$\text{WC}_{(DW \text{ basis})} = \frac{FW - DW}{DW} \times 100$$
$$\text{WC}_{(FW \text{ basis})} = \frac{FW - DW}{FW} \times 100$$

Molecular investigation assay

RNA isolation

RNA was extracted according to the manufacturer's instructions for the RNA kit (Geneaid, Taiwan).

Single-strand DNA synthesis

After RNA isolation, the cDNA synthesis phase was started. Amplification mixture (GeneAll, S.Korea) was composed of 10X Reaction Buffer 2 µl, 20X dNTP (1µl), Rta-se (1µl), RNase Inhibitor (0.5µl), RNase free Water (3.5µl), RNA Sample (6µl), primers (3µl) and RNA Sample (10µl). Amplification steps included 25°C (10min), 27°C (120min) and 85°C (5min).

Real-Time qPCR profile

Amplification mixture was used in this step composed of 2X MasterMix (S YBR Green) 10µl, primers 2µl, DNA template 2µl and RNase free distilled water 6 µl (GeneAll, S. Korea), primers that used were, wheat growth indicator, Ta-GA3ox2-1-S-5'-GTACATGGCGTGCAGCAAGAAG-3', Ta-GA3ox2-1-A-5'-GCACGCATCC ACCAGCATCATC-3 (26), spike development, Ta14S-R1-5'-CGCCT GCTAC-GCTACAAGGAC-3', Ta14S-F2- 5'-GTCAATGACCGTTG CAATGTG3', in addition to β-actin-F-5'-TTTGAAGAGT-CGGTGAAGGG-3', β-actin-R-5'-TTT CATAACAGCAGGCAA-GCA-3'¹⁷. Amplification mixture plate was placed in Applied Biosystem 7500 fast thermocycler; an amplification program was included, initial unwinding at 95°C (5min), then 40 cycles of unwinding at 95 °C (30 sec), primer hybridization at 60°C, and 57°C (30sec) respectively and extension 72°C (30sec).

Statistical analysis

Phenotypic outcomes analysis

The data were statistically evaluated by SPSS Program, and differences in treatment means were compared using the Completely Randomize Design (CRD) test at a probability level of 0.05²³.

Molecular outcomes analysis

The quantification findings were expressed in terms of the cycle threshold value calculated using the manually modified baseline. The method mentioned earlier was used to determine relative gene expressions. To detect the difference between control and treated cases, differences in the amplification values of target and reference genes were estimated as expression levels (fold change) for each sample^{18,24}.

Results

The current findings (table1) revealed a substantial difference in wheat germination growth characteristics after

being treated with an aqueous solution of three weed residues(5%,w/v). According to the mean effect of cultivar, the results showed the highest effect of seed germination percent in IPA-99(91.67%). After treatment with *Malva parviflora* L. aqueous extracts, the highest increase in seed germination percent (96.67%) was reported in the Rashid cultivar. According to the mean effect of weeds, the highest seed germination percent (93.33%) was noticed after treatment with *Malva parviflora* L.

Regarding the mean effect of cultivar, the outcomes revealed the highest impact of plumule length in Abo Garib (4.38cm). The treatment with *Silybum marianum* L., aqueous extracts showed the highest increase in plumule length (4.93cm) reported in the Abo Garib cultivar. The means effect of weeds showed the highest value in plumule length (4.04cm)after treatment with *Malva parviflora* L.

The mean effect of cultivar outcomes showed the highest impact of plumule weight in Rashid(0.07gm). Due to treatment with *Silybum marianum* L., aqueous extracts' highest increase in plumule weight(0.092gm) was documented in the Ras-hid cultivar. The mean effect of weeds showed the highest value in plumule weight (0.054gm)after treatment with *Silybum marianum* L. The mean effect of cultivar outcomes showed the highest impact of radical length in Abo Garib (6.19cm). Because of therapy with *Lolium rigidum* L., aqueous extracts, the highest reducing effect of radical length(2.97cm) was noticed in the Rashid cultivar. The mean effect of weeds showed the highest value in radical length (4.93 cm)after treatment with *Silybum marianum* L.

The mean effect of cultivar outcomes showed the highest effect of radicale weight in Rashid(0.039gm).because of treatment with *Silybum marianum* L.,aqueous extracts, the highest increasing effect of radicale length(0.045cm)was noticed in Abo Garib cultivar. The mean impact showed the highest value in weight(0.038gm)after treating with *Lolium rigidum* L.

Figure 1. Shows that the three weed residues were affected in the seed germination percent of three wheat cultivars. The highest increasing value was linked with Abo

Ghraib cultivar after cultivation in the soil incubated with *Silybum marianum* L. and *Malva parviflora* L. residues. In contrast, the highest reduction percent of IPA99 seed germination was documented after cultivation in soil with *Silybum marianum* L. and *Malva parviflora* L.

The three cultivars that were cultivated in the soils incubated with weeds residues showed variant increasing values in spike length. However, the remainder of the treatments varied in spike length with different cultivars; the highest spike length was linked with Abo Garib after treatment with *Malva parviflora* L., as represented in the histogram (figure2).

Figure (3) demonstrated a variance in the number of leaves of the three wheat cultivars cultivated in the weed-containing soil compared to the number of plants grown in the control soil(without weeds). The number of leaves of the cultivar IPA99 treated with *Lolium rigidum* L. revealed the highest increasing value.

The present results demonstrated that the weight of 100grains was differed according to the three wheat cultivars after treated with soil weed residues. Cultivar Rashid treated with soil having *Malva parviflora* L. residues showed the most significant value of growth (figure 4).

In the soil incubated with weed residues, there were changes in the relative water content in wheat cultivars, as shown in Figure(5). The relative water content of Abo Ghraib plants grown in soils incubated with *Malva parviflora* L. residues showed the highest increasing value.

Relationship between phenotypic and genotypic results

This study showed significant positive predictive values as phenotypic results associated with specific molecular outcomes(table2). In term of wheat length, there was a significance increasing percentage in Rashid cultivar after treat-ing with *Lolium rigidum* L. and *Malva parviflora* L.(L-Treated(T),45cm: control (c),34cm and M-treat-d,44cm: control,34cm resp.), these results were confirmed

Cultivars	Treatment	Seed germination (%)	Plumule length (cm)	Plumule Fresh weight (gm)	Radical Length (cm)	Radical fresh weight (gm)
Rashid	Control	80.00 d	4.0 ab	0.040 c	7.10 ab	0.038 cd
	<i>Lolium rigidum</i> L.	90.0 bc	4.93 a	0.074 b	8.50 a	0.063 a
	<i>Silybum marianum</i> L.	83.33 cd	2.83 de	0.092 a*	2.97 d*	0.026 fgh
	<i>Malva parviflora</i> L.	96.67 ab*	3.80 ae	0.075 b	6.17 abc	0.03 ef
	Mean cultivars effect	87.50 b	3.89 a	0.070 a	6.18 a	0.039 a
Abo Gharib	Control	83.33 cd	3.60 b-e	0.019 f	7.53 ab	0.019 i
	<i>Lolium rigidum</i> L.	83.33 cd	4.33 abc	0.040 c	5.30 bc	0.029 efg
	<i>Silybum marianum</i> L.	86.67 cd	4.93 a*	0.041 c	6.40 abc	0.045 b*
	<i>Malva parviflora</i> L.	83.33 cd	4.63 ab	0.041 c	5.53 bc	0.026 fgh
	Mean cultivars effect	84.17 b	4.38 a	0.035 b	6.19 a	0.029 b
IPA 99	Control	86.67 cd	3.23 de	0.030 d	5.67 bc	0.033 de
	<i>Lolium rigidum</i> L.	100.0 a	2.60 e	0.029 e	4.60 cd	0.023 hi
	<i>Silybum marianum</i> L.	80.0 d	3.37 cde	0.029 e	5.43 bc	0.024 ghi
	<i>Malva parviflora</i> L.	100.0 a	3.70 b-e	0.039 c	6.33 abc	0.039 c
	Mean cultivar effect	91.67 a	3.23 b	0.031 c	5.51 a	0.029 b
Mean weeds effect	Control	83.33 b	3.61 a	0.029 c	6.77 a	0.030 b
	<i>Lolium rigidum</i> L.	91.11 a	3.96 a	0.046 b	6.13 ab	0.038 a
	<i>Silybum marianum</i> L.	83.33 b	3.71 a	0.054 a	4.93 b	0.031 b
	<i>Malva parviflora</i> L.	93.33 a	4.04 a	0.052 a	6.01 ab	0.032 b

Green color, highest increasing value; red color, highest decreasing value and*, highest weed effect.

Table 1. Effect of aqueous extracts of weeds in germination and growth of three wheat cultivars.



Figure 1. Histogram of seed germination of three cultivars of wheat plants treated with three weeds (*Silybum marianum* L., *Malva parviflora* L., and *Lolium rigidum* L.).

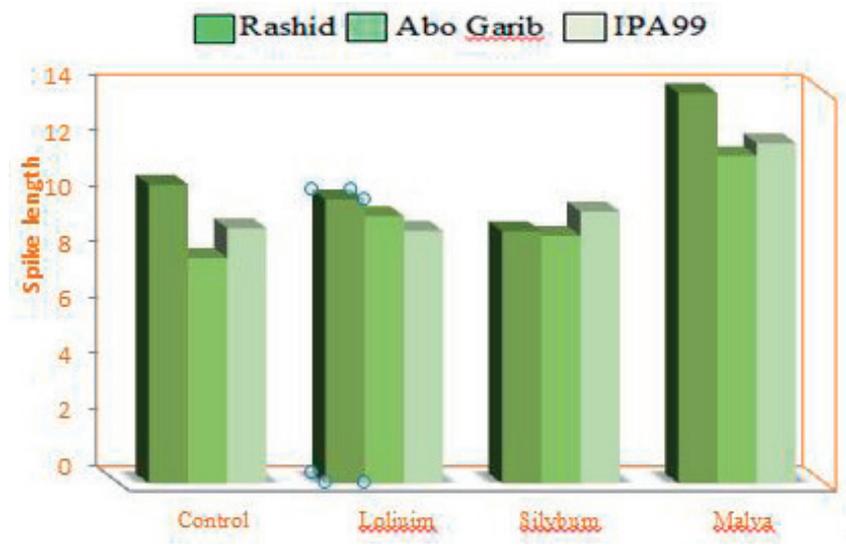


Figure 2. Histogram of spike length of three cultivars of wheat plants treated with three weeds (*Silybum marianum* L. and *Malva parviflora* L. and *Lolium rigidum* L.).

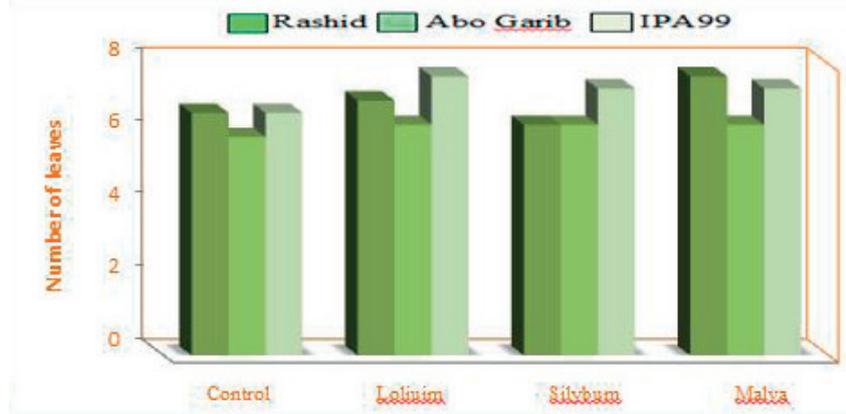


Figure 3. Histogram of the number of leaves of three cultivars of wheat plants treated with three weeds (*Silybum marianum* L. and *Malva parviflora* L. and *Lolium rigidum* L.).

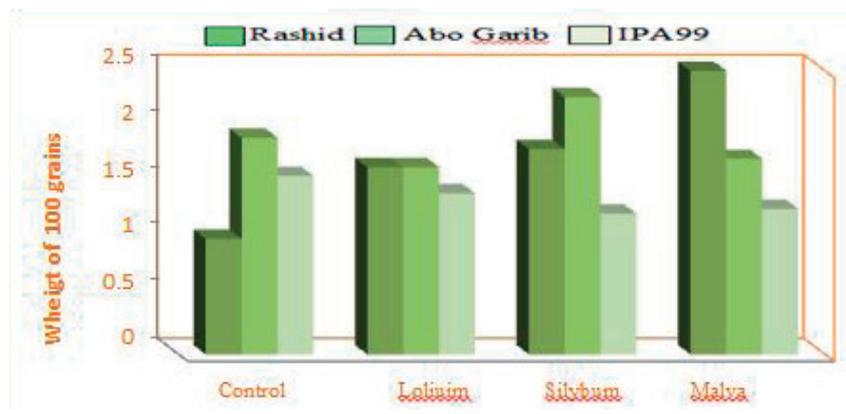


Figure 4. Histogram of the weight of 100 grains of three cultivars of wheat plants, treated with three weeds (*Silybum marianum* L. and *Malva parviflora* L. and *Lolium rigidum* L.).

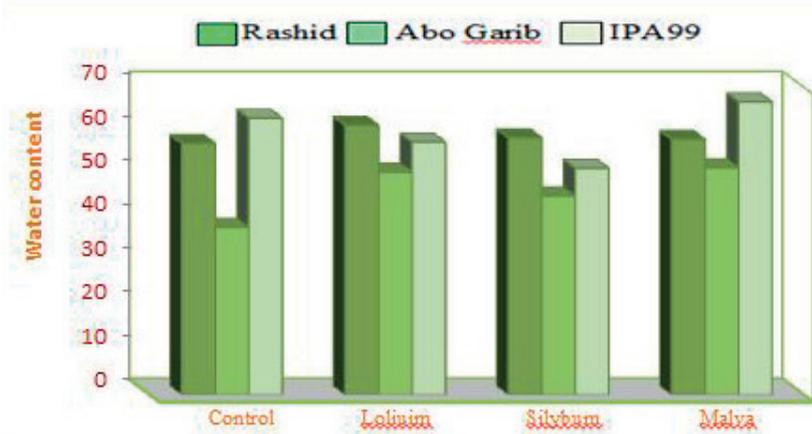


Figure 5. Histogram of the water content of 100 grains of three cultivars of wheat plants treated with three weeds (*Silybum marianum* L. and *Malva parviflora* L. and *Lolium rigidum* L.).

with TaGA3ox2-1 gene expression related with the same case (L-t,5.1043:c,0.870 67 and M-t,3.96014:c,0.87067 resp.) other outcomes showed high expression with previous gene (T,7.96787:c,0.942034), that associated with phenotypic finding (T, 55:c,35) after treating of Abo Grib cultivar with *Malva parviflora* L. as well as, growth value was increased in IPA99 cultivar (T,44:c,21), which agreed with that documented genotypically (T,6.85702:c,1.01002). Spike weight showed the highest increasing value phenotypically in Rashid (t,55gm:c35, gm) after treating Abo Grib cultivar with *Malva parviflora* L., which in turn agreed with Ta14S-R1 gene expression (T,6.71807:c,1.01065).

Discussion

Weeds leave massive amounts of residue in the field, affecting the linked crops and subsequent crops in various cropping systems. Allelochemicals released by weeds impact agricultural plant germination, stand establishment, growth, yield, and physiology. By regulating enzyme activity, protein synthesis, photosynthesis, respiration, cell division, and enlargement, among other physiological functions, they produce significant reductions in crop germination and growth, resulting in a significant drop in crop production. Allelopathic weeds, in short, are a possible hazard to crop plants and can result in financial losses²⁵.

The present results showed a synergistic relationship between utilizing weed residues and wheat cultivars growth in which the studied parameters were increased. In turn, these outcomes agreed with the documented results of many researchers and disagreed with others. According to (26), some plant species have a more considerable inhibitory impact when used as residues due to higher allelochemical extraction during the decomposition process. *Flaveria bidentis* L., an invasive weed, was shown to exude allelopathic phenolic chemicals, and residues from this weed hindered the growth and biomass of cotton seedlings. There was the highest decrease in root fresh (64%) and dry biomass (64%) and root/shoot ratio (64%) after being treated with *V. sativa* raw residues. The outcomes showed that the interaction of leaches and residues could lead to a synergistic effect in nature. In turn, this causes significant economic and environmental disposal of waste and organic materials accumulation²⁷.

On the other hand, (28) used various concentrations of aqueous extracts (5, 10 and 15g/l) of the three weeds, significantly reducing the percent germination of Pirsabaq cultivar. In pot culture, root and shoot length and dry seedling biomass of the three wheat varieties showed differential responses to different weeds. Aqueous extract at 15g/l of *A. fatua* increased the source, shot height, and dry weight of Pirsabaq cultivar¹¹.

Phenotypic investigation (Greenhouse)				Molecular screening (RTqPCR)				
Control	<i>L. rigidum</i>	<i>S. marianum</i>	<i>M. parviflora</i>	Gene	Control	<i>L. rigidum</i>	<i>S. marianum</i>	<i>M. parviflora</i>
1.25	1.13	0.85	3.0	Ta GA3ox2-1	1.01078	0.94783	0.95015	4.21653
0.65	2.05	0.98	0.91		0.94891	3.01892	1.40132	0.76258
1.20	1.21	0.70	2.50		1.78756	2.20189	0.90143	4.10397
34	45	35	44	Ta14S-R1	1.00912	5.01891	2.10442	3.95403
35	45	42	55		1.01065	4.88201	4.01627	6.71807
21	35	33	44		0.81223	1.77189	1.53041	5.10176

Table 2. Comparison between phenotypic results and molecular screening outcomes.

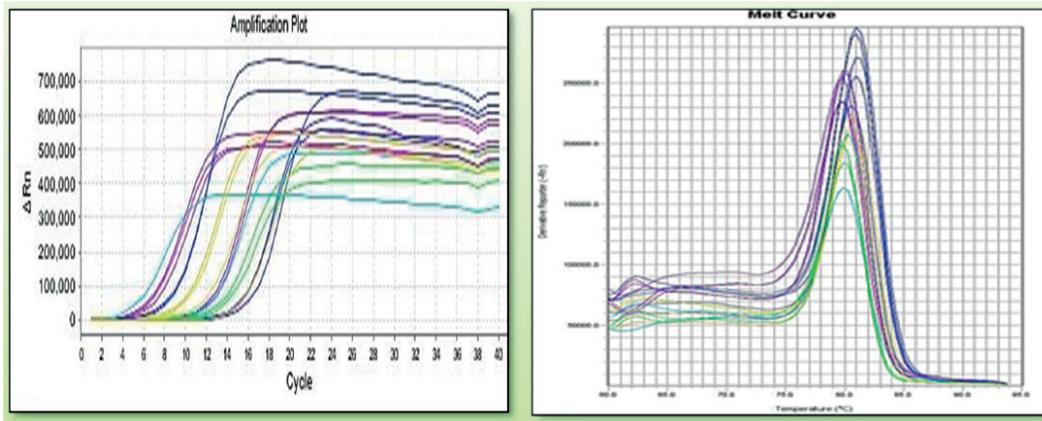


Figure 6. Shows the events of the *TaGA3ox2-1* gene amplification curve via RTqPCR, in which different values of previous gene expression were documented.

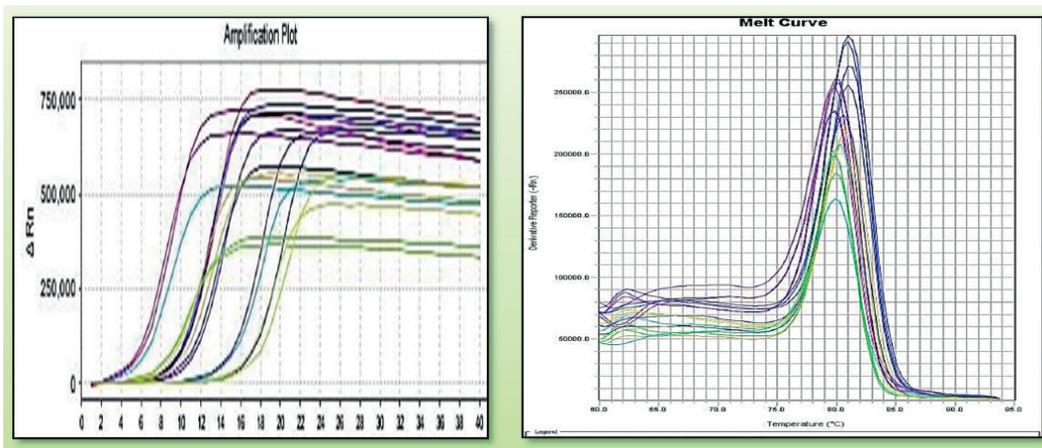


Figure 7. Comparative curves of *TaGA3ox2-1* gene. Right, amplification curves and left melting curves.

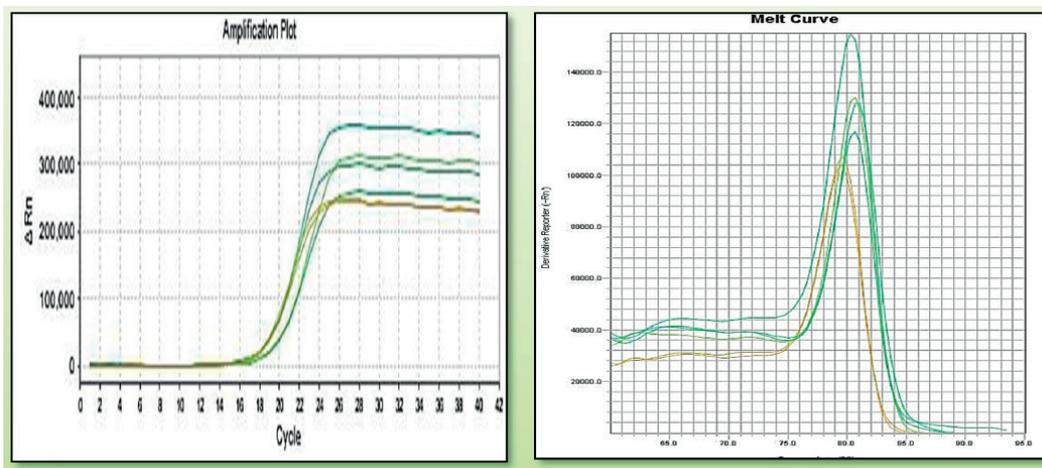


Figure 8. Showed the events of *B-actin* gene amplification curve via RTq-PCR as a reference gene.

Conclusions

In terms of molecular investigation, many studies were achieved in this field over the world²² noticed that among the 18 genes analyzed, genes encoding enzymes that promote the synthesis of bioactive gibberellin like *TaGA20ox-2*, *TaGA20oxD*, *TaGA3ox2-1*, *TaGA3ox2-2*, *TaGA3ox2-2*, these genes act as positive components in its response pathways were up-regulated in hybrid cultivars. A previous study showed that three *Ta14S* homoeologous genes have regu-

latory roles in seed development and germination via synthesis of 14-3-3 proteins are involved in signal transduction pathways with significant roles in late embryo development in seeds and germination, which represented a high transcription of *Ta14S-2B*²⁹.

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Conflicts of Interest

There is no conflict.

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