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Investigating the Phenophysiological Traits of Transgenic Rapeseed Lines (*Brassica napus*) with *aro*A Gene Harboring a Point Mutation of Proline 101 to Serine (P101S) under Glyphosate Herbicide Treatment

Amir Roeintan[1], Seyed Mehdi Safavi[*1], Danial Kahrizi[2]

[1]*Department of Agronomy and Plant Breeding, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran*
[2]*Agricultural Biotechnology Department, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran*

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ABSTRACT

One of the most effective ways to breed glyphosate-resistant plants is to reduce the affinity of glyphosate to the EPSPS enzyme by manipulating the *aro*A gene. In this study, P101S mutation was induced in the *aro*A of *E. coli* and the mutant gene was cloned in the pUC18 plasmid. It was transferred by *Agrobacterium tumefaciens* to the rapeseed. To investigate the phenophysiological traits in transgenic rapeseed lines under different glyphosate treatments (0, 1.2, 2.4, 4.8, 9.6, 19.2, 38.4, 76.8 and 153.6 mM), the seeds of T_2 generation transgenic plants were studied in greenhouse conditions in a factorial experiment. The results showed that the control (no herbicide) had the lowest amount of days to flowering (32.5 days), partial water pressure (14.92kPa), active photosynthetic radiation (393.4 mmol m^{-2} s^{-1}), and leaf surface temperature (28.880°C). The concentration of 2.4 mM had the highest stomatal conductance (0.573 mol m^{-2} s^{-1}) and photosynthesis rate (14.07 µmol m^{-2} s^{-1}). The lowest value of stomatal resistance was related to the concentration of 4.8 mM (246.9 mmol m^{-2} s^{-1}), and the lowest value of stomatal conductance was associated with 9.6 mM (0.047 mol m^{-2} s^{-1}). The highest rate of CO_2 source (416.3 ppm) and active photosynthetic radiation (576.9 mmol m^{-2} s^{-1}) was seen in the 19.2 mM. The 38.4 mM had the highest number of days to flowering (48.1 days) and leaf surface temperature (38.748°C) and the lowest amount of CO_2 source (386ppm). The 76.8 mM had the highest stomatal resistance (330.2 mmol m^{-2} s^{-1}) and the lowest photosynthesis rate (1.36 µmol m^{-2} s^{-1}). The highest partial pressure of the water source was related to 153.6 mM (19.07 kPa). In summary, different concentrations of herbicides exhibit varying degrees of phenophysiological traits, and desired traits can be improved based on these concentrations.

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1. Introduction

Rapeseed (*Brassica napus* L.) provides 12% of the world's edible oil and is considered the most important oil plant after soybean and oil palm (Chmielewska *et al.*, 2021). The value of rapeseed oil is due to the presence of unique fatty acids, which makes it more desirable than other vegetable oils (Barzan *et al.*, 2015). There are limitations in the cultivation of this valuable crop, including that weeds cause a quantitative and qualitative decrease in its yield. For this reason, weed control in rapeseed fields is one of this plant's most critical agricultural operations. Chemical herbicides are the primary method of weed control (Gaba, 2016). A wide range of weeds threaten the fields of this plant, so it is necessary to use a broad-spectrum herbicide (Gomes *et al.*, 2016). But, in addition to weeds, such herbicides also damage crops (Lemerle *et al.*, 2017). For this reason, producing rapeseed plants tolerant to these herbicides is vital (Tang *et al.*, 2019). Glyphosate is a broad-spectrum, non-selective herbicide commonly used to control weeds. This herbicide inhibits the enzyme 5-enol-Pyruvyl-Shikimate-3-Phosphate Synthase (EPSPS), the sixth enzyme in the biosynthesis pathway of cyclic amino acids (Amrhein *et al.*, 1983). Whenever this enzyme is not produced, the plant will die (Kahrizi, 2014). One of

[*] Corresponding author.
E-mail address: mehdisafavi@gmail.com

the most important ways to make crops resistant to the herbicide glyphosate is the production of EPSPS enzyme resistant to the herbicide by creating a point mutation (Devine and Shukla, 2000). Double mutation has been used to produce herbicide-resistant rapeseed (Kahrizi *et al.*, 2007). In other reports, Kahrizi and Salmanian (2008) and Palma-Bautista et al. (2023) used single mutation constructs for this purpose.

2. Materials and methods

The research was carried out in the research laboratory of Zagros BioIdea Company, Kermanshah, Iran.

2.1. Bacterial strains, vectors, and plant materials, preparation of genomic DNA from E. coli (K12) bacteria, and genetic engineering measures

Bacterial strains, vectors, and plant materials, preparation of genomic DNA from *E. coli* (K12) bacteria, and genetic engineering measures along with the evaluation of resistance to the glyphosate herbicide in transgenic plants have been previously reported in the previous work of Roeintan et al. (2022), which was carried out on the *aro*A mutant gene, including the conversion of proline number 101 to serine (P101S) (Roeintan *et al.*, 2022).

2.2. Glyphosate herbicide treatment on transgenic plants in the greenhouse

This study was conducted in 9 transgenic rapeseed lines of the T_2 generation and one control line in the greenhouse of the Faculty of Agriculture and Natural Resources, Razi University, with environmental conditions of 16 hours of light and 8 hours of darkness, a temperature of 25°C and humidity of 78-80% to investigate the level of resistance to the herbicide glyphosate. For this purpose, 360 plastic pots were prepared and filled with a mixture of field soil, sand, and cocopeat. Then, six T_2 generation rapeseed seeds from each transgenic line were planted inside each pot (the seeds were planted in four replications with the control plant). The pots were watered every day. Then, for one week after planting, all the seeds germinated, and at the four-leaf stage, different concentrations (0, 1.2, 2.4, 4.8, 9.6, 19.2, 38.4, 76.8 and 153.6 mM) of glyphosate herbicide were used for spraying. Then, the characteristics of burning percentage, photosynthesis rate, budding, plant height, number of pods in the main branch, and number of pods in the secondary branch in the transgenic and control seedlings were measured (Fig. 1).

2.3. Evaluation under greenhouse conditions

In this experiment, 270 plastic pots were filled with a mixture of field soil, sand, and cocopeat. Then the T_2 generation rapeseed seeds were planted in pots with three replications and sprayed with different concentrations of glyphosate herbicide (0, 1.2, 2.4, 4.8, 9.6, 19.2, 38.4, 76.8 and 153.6 mM) at the four-leaf stage of the plants, then the phenological and physiological traits (including budding time, water evaporation pressure, CO_2 source, photosynthetic active radiation, leaf surface temperature, stomatal conductance, photosynthesis rate, and stomatal resistance) were compared in these plants. Physiological traits were evaluated by a photosynthesis meter (Lycor 6400 model).

2.4. Statistical analysis

The experiment was conducted as a factorial in a completely randomized design with three replications. Factors include different concentrations of glyphosate and rapeseed transgenic lines. Analysis of variance and mean comparison were used for statistical analysis. The LSD method was used to compare the mean and the significance level was considered to be 5%.

3. Results and discussion

3.1. Budding time

Because some lines did not flower in some concentrations of glyphosate herbicide, the design was removed from the factorial test mode. An analysis of variance and mean comparison for each level of herbicide was performed for the remaining lines. In the condition without herbicide application, all the lines had to bud. Then, the data were analyzed based on a completely random design, and all 9 transgenic lines showed survival at concentrations of 0, 1.2, 2.4, 4.8, 9.6, 19.2, and 38.4 mM. Their analysis was done based on the factorial test. Lines 5, 8, and 9 showed survival at a concentration of 76.8 mM, and their analysis was done based on a separate, utterly random experiment. At the concentration of 153.6 mM, none of the lines showed survival, and no statistical analysis was done for it.

Figure 1. Analysis of resistance to glyphosate in transgenic rapeseed plants. A: A resistant plant. B: Sensitive plant. C: Resistant plant in flowering stage. D: treated control plants. E: Resistant transgenic plants

3.1.1. Variance analysis of line effect on the number of days to budding in conditions without herbicide application (control)

The results of the analysis of variance showed that there is a significant difference between the studied lines for the number of days to the budding trait. Because the number recorded for each line is the same in all three replications, the standard error (S.E) and the coefficient of variation (C.V) were zero. So there will be a significant difference between all the lines for this attribute. Therefore, there is no need to compare means in a particular way; only means can be presented (Table 1).

3.1.2. Variance analysis of line effect on the number of days to budding at concentrations up to 38.4 mM

All 9 transgenic lines showed survival in concentrations of 0, 1.2, 2.4, 4.8, 9.6, 19.2, and 38.4 mM, and their analysis was done based on the factorial test. The analysis of variance showed a very significant difference between the transgenic lines in the mentioned herbicide concentrations and their mutual effects for the trait of the number of days to budding. Because the number recorded for each line is the same in all three replications, the standard error (S.E) and the coefficient of variation (C.V) were zero. Therefore, there will be a significant difference between all the lines, the concentrations of herbicide used, and their mutual effects for this trait. Therefore, there is no need to compare the mean in a particular way; only the means can be presented (Table 2).

3.1.3. The mean line for the trait of days to budding under the conditions of glyphosate herbicide application

Considering that there is no difference between the investigated repetitions for each line and each concentration, but there is a difference between different concentrations, the standard error for comparing the lines cannot be zero, and the difference between the lines is significant. By examining the means (Table 3), it is clear that line 1 showed the highest amount of days to flowering (49.4 days), and line 8 led the lowest amount of days to flowering (35.3 days).

Table 1. Variance analysis of line effect on the number of days to budding in conditions without herbicide application

Source of Variation	D.F.	Mean Square
Line	9	170.7**
Experimental Error	20	0.00
CV (%)	-	0.00

** means significant at a 1% probability level

Table 2. Variance analysis of the line effect on the number of days to budding at zero to 38.4 mM concentration

Source of Variation	D.F.	Mean Square
Line	8	9.194**
Herbicide Concentration	6	5249.0**
Line × Herbicide Concentration	48	3666.0**
Experimental Error	126	0.00
CV (%)	-	0.00

** means significant at a 1% probability level

Table 3. Mean comparison of lines for the number of days to budding under the conditions of glyphosate herbicide application (from 0 to 38.4 mM)

Line number	Days to Budding	Standard Error
1	49.4	1.46
2	37.5	2.06
3	38.5	1.40
4	43.8	1.61
5	39.7	1.90
6	37.5	1.32
7	37.4	1.49
8	35.3	0.39
9	37.7	1.56
Total	39.6	0.58

3.1.4. Mean herbicide concentration for the number of days to budding trait

Considering that there was no difference between the investigated repetitions for each line and each concentration, but there was a difference between different lines, the standard error for comparing herbicide concentrations cannot be zero, and the difference between concentrations is significant. According to Table 4, it is clear that the concentration of 38.4 mM has the highest number of days to flowering, with 1.48 days, and the concentration of 0 mM has the lowest number of days to flowering, 32.5. The correlation coefficient between the herbicide concentration and the number of days to budding was estimated as positive and significant (0.88). It shows that the increase in herbicide concentration increases the length of the plant's growing period, and the plant enters the reproductive phase later. Also, the coefficient of explanation was calculated as 0.78, indicating that 78% of the changes in the number of days to budding depend on the herbicide concentration, and 22% depends on other factors. In the regression analysis, it was found that the regression coefficient of the effect of herbicide concentrations on the trait number of days to budding is positive and significant (0.36), and the width from its origin is also positive and significant (35.7).

Table 4. Mean comparison of herbicide concentrations for the number of days to budding trait

Concentration (mM)	Days to Budding	Standard Error
0	32.5	0.35938
1.2	37.8	1.56469
2.4	39.1	1.09951
4.8	35.5	1.09041
9.6	37.8	1.24531
19.2	46.6	1.14727
38.4	48.1	1.37575
Total	39.6	0.58315

3.1.5. Investigating the mean interaction effect of line and herbicide concentration for the trait of number of days to budding

According to Suppliment Table 1 (Table S1), the highest number of days until budding (55 days) corresponds to the concentration of 38.4 mM in lines 4 and 5 and the lowest number of days until budding (31 days), seen in most lines in minimum herbicide concentrations. On the other hand, because the number recorded for each line is the same in all three repetitions, the standard error (S.E.) was zero. So there will be a significant difference between all interaction effects for this trait. Therefore, there is no need to compare means in a particular way; only means can be presented.

3.1.6. Variance analysis of line effect on day to budding at 76.8 mM concentration

Transgenic lines 5, 8, and 9 showed survival in the concentration of 76.8 mM of herbicide, and analysis of these lines was done based on a completely random design. The results of the analysis of variance showed that there is a significant difference between the transgenic lines for the number of days to the budding trait. Because the number recorded for each line is the same in all three repetitions, the standard error (S.E) and the coefficient of variation (C.V) were zero (Table S2). So there will be a significant difference between all the lines for this trait. Therefore, there is no need to compare means in a particular way; only means can be presented.

3.1.7. The mean of the lines for the trait of day-to-budding in concentrations of 76.8 mM of glyphosate herbicide

Considering that there is no difference between the investigated repetitions for each line and each concentration, the standard error for comparing the lines was zero, and the difference between the lines is significant. By examining the means, it is clear that lines 5 and 9 have the highest number of days to flowering (54 days), and line 8 has the lowest number of days to flowering (34 days) (Table S3).

3.2. Water evaporation pressure (Eref)

Transpiration means converting water into steam through the plant's stomata and leaving it. Transpiration occurs when the water vapor pressure inside the plant is higher than the vapor pressure surrounding the plant. Since no seedling was produced for the desired trait in several herbicide concentrations, the experimental design was analyzed as unbalanced. Table S4 shows the number of seedlings used in each concentration and line for this trait.

3.2.1. Variance analysis table of line effect and glyphosate herbicide concentration on water vapor pressure trait

Variance analysis Table S5 shows that the effect of line, the effect of herbicide concentration and the interaction effect of line and herbicide concentration on water evaporation pressure are very significant.

3.2.2. Comparison of the mean effect of glyphosate concentration on water source partial pressure

A comparison of the mean effect of herbicide concentration on the partial pressure attribute of the water source shows that this attribute significantly differs in different herbicide concentrations. The highest partial pressure of the water source corresponds to a concentration of 153.6 mM (19.07kPa), and the lowest one corresponds to a concentration of 0 mM (14.92 kPa). As it is known, the partial pressure of the water source increases with the increase in herbicide concentration, and the correlation coefficient between herbicide concentration and the target trait was estimated as positive and highly significant (0.65). Also, the coefficient of explanation was calculated as 0.78, which indicates that 43% of the changes of this trait depend on the herbicide concentration and 57% of it depends on other factors. The regression analysis found that the regression coefficient between herbicide concentrations and the target trait is positive and significant (0.47), and the width from its origin is also positive and significant (14.97).

3.2.3. Investigating the mean interaction effect of line and herbicide concentration for partial pressure trait of water source

According to Table S6, the interaction effect of line and herbicide concentration on the partial pressure attribute of the water source is significant. The highest partial pressure of the water source corresponds to the concentration of 76.8 mM in line 1 (19.50 kPa). The lowest value of this trait corresponds to the concentration of 1.2 mM in line 3 (13.27 kPa).

3.3. CO_2 source (Cref)

Carbon is one of the elements plants require, and its amount is higher than other elements. Nearly 40% of the dry weight of the plant is made up of carbon. Plants get the carbon they need from carbon dioxide in the air. Most of this gas enters the plant through the leaves. When the carbon dioxide gas enters the plant cells, it is converted into carbon hydrate with the help of sunlight energy and is transferred to other parts of the plant. Since no seedling was produced for the desired trait in some herbicide concentrations, the design was analyzed as unbalanced. Table S7 shows the number of seedlings used in each concentration and line for this trait.

3.3.1. Variance analysis table of line effect and glyphosate herbicide concentration on CO_2 source traits

The variance analysis Table S8 shows that the effect of line and herbicide concentration on the trait of CO_2 source is very significant, and the interaction effect of line and herbicide concentration on this trait is significant.

3.3.2. Comparison of the mean effect of glyphosate herbicide concentration on CO_2 source traits

A comparison of the mean effect of herbicide concentration on the CO_2 source trait shows that this trait significantly differs in different herbicide concentrations. Its highest value corresponds to the concentration of 4.8 mM (398.59 ppm), and its lowest value corresponds to the concentration of 38.4 mM (387.89 ppm). The correlation coefficient between the herbicide concentration and the desired trait was estimated as negative and non-significant (-0.61), which indicates the absence of a relationship between these two variables.

3.3.3. Investigating the mean interaction effect of line and herbicide concentration for the trait of CO_2 source

According to Table S9, the interaction effect of line and herbicide concentration on the trait of CO_2 source is significant. The highest value of this trait is related to the concentration of 19.2 mM in line 5 (416.3 ppm). The lowest value of this attribute corresponds to the concentration of 38.4 mM in lines 4 and 5 (386 ppm).

3.4. Photosynthetic active radiation (Parleaf)

The wavelength between 400-700 nm, directly absorbed by chlorophyll and essential in photosynthesis, is called photosynthetic active radiation. Since no seedling was produced for the desired trait in several herbicide concentrations, the design was analyzed as unbalanced. Table S10 shows the number of seedlings used in each concentration and line for this trait.

3.4.1. Variance analysis table of the effect of line and glyphosate herbicide concentration on photosynthetic active radiation trait

The variance analysis table (Table S11) shows that the effect of line, the effect of herbicide concentration, and the interaction impact of line and herbicide concentration on the trait of photosynthetically active radiation are very significant.

3.4.2. Comparison of the mean effect of glyphosate concentration on active photosynthetic radiation

A comparison of the mean effect of herbicide concentration on the photosynthetic active radiation trait shows that this trait significantly differs in different concentrations of rapeseed. Its highest value corresponds to the concentration of 19.2 mM (576.9 mmol m^{-2} s^{-1}), and its lowest value corresponds to the concentration of 0 mM (393.4 mmol m^{-2} s^{-1}). The correlation coefficient between herbicide concentration and photosynthetically active radiation trait was estimated as negative and non-significant (-0.163), which indicates the absence of a relationship between these two variables.

3.4.3. Investigating the mean interaction effect of line and herbicide concentration for photosynthetically active radiation trait

Table S12 shows the interaction effect of line and herbicide concentration on photosynthetic active radiation trait is significant. The highest value of this trait corresponds to the concentration of 4.8 mM in line 9 (617.6 mmol m^{-2} s^{-1}). The lowest value of this attribute is related to 0 mM concentration in line 2 (268.6 mmol m^{-2} s^{-1}).

3.5. Leaf surface temperature (Tleaf)

Since no seedling was produced for the desired trait in some herbicide concentrations, the design was analyzed as unbalanced. Table S13 shows the number of seedlings used in each concentration and line for this trait.

3.5.1. Variance analysis of line effect and glyphosate herbicide concentration on leaf surface temperature

Table S14 shows the results of the leaf surface temperature variance analysis that the effect of line, the impact of herbicide concentration, and the interaction effect of line and herbicide concentration on this trait is very significant.

3.5.2. Comparison of the mean effect of herbicide concentration for leaf surface temperature

A comparison of the mean effect of herbicide concentration on leaf surface temperature trait shows that this trait has a significant difference in different concentrations of herbicide, and its highest value is related to the concentration of 38.4 mM (38.748°C) and the lowest value is associated with the concentration of 0 mM (28.880°C). The concentrations of 2.4, 76.8, and 153.6 are not significantly different. The temperature of the leaf surface increases with the increase in herbicide concentration. The correlation coefficient between the herbicide concentration and the desired attribute was estimated as positive and non-significant (0.159).

3.5.3. Investigating the mean interaction effect of line and herbicide concentration for the trait of leaf surface temperature

Table S15 shows the interaction effect of line and herbicide concentration on leaf surface temperature is significant. The highest value of this attribute is related to the concentration of 19.2 mM in line 9 (39.63°C). The lowest value of this attribute is associated with the concentration of 0 mM in line 8 (26.28°C).

3.6. Stomatal conductance (GS)

Since no seedling was produced for the desired trait in a number of herbicide concentrations, the design was analyzed as unbalanced. Table S16 shows the number of plants used in each concentration and line for this trait.

3.6.1. Variance analysis of line effect and glyphosate herbicide concentration on stomatal conductance trait

The analysis of the variance table (Table S17) shows that the effect of the line the effect of herbicide concentration on the stomatal conductance trait is very significant. Still, the interaction effect of line and herbicide concentration on this trait is insignificant.

3.6.2. Comparison of the mean effect of herbicide concentration on stomatal conductance

A comparison of the mean effect of herbicide concentration on the stomatal conductance trait shows that this trait significantly differs in different herbicide concentrations. Its highest value corresponds to a concentration of 2.4 mM (0.381 mol m^{-2} s^{-1}), and its lowest value corresponds to a concentration of 153.6 mM (0.028 mol m^{-2} s^{-1}). As it is known, stomatal conductance decreases with the increase in herbicide concentration, and the correlation coefficient between herbicide concentration and the target trait was estimated as negative and significant (-0.67).

3.6.3. Investigating the mean interaction effect of line and herbicide concentration for stomatal conductance trait

Table S18 shows the interaction effect of line and herbicide concentration on stomatal conductivity is significant. The highest value of this attribute corresponds to the concentration of 2.4 mM in line 3 (0.573 mol m^{-2} s^{-1}). The lowest value of this attribute corresponds to the concentration of 9.6 mM in line 4 (0.047 mol m^{-2} s^{-1}).

3.7. Photosynthesis rate (A)

Since no seedling was produced for the desired trait in several herbicide concentrations, the design was analyzed as unbalanced. Table S19 shows the number of seedlings used in each concentration and line for this trait.

3.7.1. Variance analysis of line effect and glyphosate herbicide concentration on photosynthesis rate trait (A)

The variance analysis table (Table S20) shows that the effect of line and the effect of herbicide concentration on the photosynthesis rate trait are very significant, and the interaction of line and herbicide concentration on this trait is significant.

3.7.2. Comparison of the mean effect of herbicide concentration on photosynthesis rate trait

A comparison of the mean effect of herbicide concentration on the photosynthesis rate trait shows that this trait significantly differs in different herbicide concentrations. Its highest value corresponds to the concentration of 2.4 mM (9.48 μmol m^{-2} s^{-1}), and its lowest value corresponds to the concentration of 153.6 mM (2.21 μmol m^{-2} s^{-1}). The rate of photosynthesis decreases with the increase in herbicide concentration. The correlation coefficient between herbicide concentration and photosynthesis rate was estimated as negative and significant (-0.84).

3.7.3. Investigating the mean interaction effect of line and herbicide concentration for photosynthesis rate trait

Table S21 shows the interaction effect of line and herbicide concentration on the photosynthesis rate trait is significant. The highest value of the photosynthesis rate corresponds to the concentration of 2.4 mM in line 9 (14.07 μmol m^{-2} s^{-1}). The lowest value of this trait corresponds to the concentration of 76.8 mM in line 1 (1.36 μmol m^{-2} s^{-1}).

3.8. Stomatal resistance (RS)

Since no seedling was produced for the desired trait in some herbicide concentrations, the design was analyzed as unbalanced. Table S22 shows the number of seedlings used in each concentration and line for this trait.

3.8.1. Variance analysis of line effect and glyphosate herbicide concentration on stomatal resistance trait

The variance analysis table (Table S23) shows that the effect of line and the interaction effect of line and herbicide concentration on the stomatal resistance trait is insignificant and the effect of herbicide concentration on this trait is very significant.

3.8.2. Mean comparison effect of herbicide concentration on stomatal resistance trait

A mean comparison of the effect of herbicide concentration on the stomatal resistance trait shows that this trait significantly differs in different herbicide concentrations. Its highest value corresponds to a concentration of 76.8 mM, (330.2 mol m^{-2} s^{-1}), and its lowest value corresponds to a concentration of 4.8 mM (246.9 mol m^{-2} s^{-1}). Different concentrations of 1.2, 2.4, and 157.6 have no significant differences, and concentrations of 38.4 and 76.8 also have no significant differences in terms of the desired trait. By increasing the herbicide concentration, stomatal resistance increases.

3.8.3. Investigating the mean interaction effect of line and herbicide concentration for stomatal resistance trait

Table S24 shows the significant interaction effect of line and herbicide concentration on the stomatal resistance trait. The highest value of this attribute corresponds to the concentration of 19.2 mM in line 1 (581.3 mol m^{-2} s^{-1}). The lowest value of this attribute corresponds to the concentration of 2.4 mM in line 2 (198.3 mol m^{-2} s^{-1}).

Rapeseed (*Brassica napus*) is considered one of the world's most important edible and industrial oilseeds. The presence of weeds in rapeseed fields is one of the most critical factors threatening its cultivation and expansion, which causes a decrease in yield and the quality of the oil obtained (Kahrizi, 2014). Therefore, the fight against weeds is one of the most essential stages of this oil plant. The central axis of the fight against weeds is chemical herbicides. Due to the wide range of weeds that threaten the fields of this plant, it is necessary to use broad-spectrum herbicides (Asaduzzaman *et al*., 2020). The main problem in using these herbicides is the sensitivity of the rapeseed plant to it. To solve this problem, producing rapeseed plants resistant to broad-spectrum herbicides such as glyphosate, which are non-selective and do not have adverse effects on humans, animals, and the environment, is essential (Tang *et al.*, 2019).

Glyphosate is included in the group of amino acid synthesis inhibitors. Glyphosate is a penetrating herbicide that destroys weeds by inhibiting a target site (Bhatt *et al.*, 2021). The herbicide disrupts the synthesis of aromatic amino acids by affecting the shikimate pathway and inhibiting the enzyme 5-Enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Leino *et al.*, 2021). The desired enzyme is necessary to produce chorismate in the shikimic acid pathway, an intermediate precursor in synthesizing aromatic amino acids and various secondary metabolites. Glyphosate causes its transfer to all meristem tissues and the death of all plant growth points, including underground growth points (Griffin *et al.*, 2021).

Since the introduction of the herbicide glyphosate, there has been a great challenge in whether or not to use it worldwide. Concerning glyphosate blowing to non-target plants is one of the problems of using this herbicide (Duke, 2021). One of the ways to create resistance to this herbicide is to produce transgenic plants resistant to this herbicide (Pan *et al.*, 2021). In this study, transgenic rapeseed lines (*Brassica napus*) with *aro*A gene having a point mutation of proline number 101 to serine (P101S) were used to evaluate the resistance of these lines against glyphosate via phenophysiological traits. The results showed that the concentration of 0mM herbicide had the lowest amount of days to flowering (32.5 days), partial water pressure (14.92kPa), active photosynthetic radiation (393.4 mmol m^{-2} s^{-1}), and leaf surface temperature (28.880°C). The concentration of 2.4mM had the highest stomatal conductance (0.573 mol m^{-2} s^{-1}) and photosynthesis rate (14.07 µmol m^{-2} s^{-1}). The lowest value of stomatal resistance was related to the concentration of 4.8mM (246.9 mmol m^{-2} s^{-1}), and the lowest value of stomatal conductance was associated with the concentration of 9.6mM (0.047 mol m^{-2} s^{-1}). The highest rate of CO_2 source (416.3ppm) and active photosynthetic radiation (576.9 mmol m^{-2} s^{-1}) was seen in the concentration of 19.2mM. The concentration of 38.4mM had the highest number of days to flowering

(48.1 days) and leaf surface temperature (38.748°C) and the lowest amount of CO_2 source (386ppm). The concentration of 76.8 mM had the highest stomatal resistance (330.2 mmol m^{-2} s^{-1}) and the lowest photosynthesis rate (1.36 μmol m^{-2} s^{-1}). Finally, the highest partial pressure of the water source was related to the concentration of 153.6 mM (19.07 kPa). In general, different concentrations of herbicide showed different levels of phenophysiological traits which can be used to improve the desired traits according to these concentrations.

4. Conclusion

Since the advent of the herbicide glyphosate, there have been huge challenges around the world in its use. One of the problems with using this herbicide is spraying glyphosate on non-target plants. One way to create resistance to this herbicide is to create genetically modified plants. In this study, transgenic rapeseed lines (*Brassica napus*) with aroA gene having a point mutation of P101S were used to evaluate the resistance of these lines to glyphosate through pheno-physiological traits. The results showed that days to flowering, water pressure, active photosynthetic radiation and leaf temperature were lowest at control herbicide concentration. Stomatal conductance and photosynthetic rate were the highest at 2.4 mM concentration. The lowest value of stomatal resistance is related to the concentration of 4.8 mM, and the lowest value of stomatal conductance is related to the concentration of 9.6mM. At the concentration of 19.2 mM, the CO2 source and active photosynthetic radiation rate were the highest. The flowering days and leaf temperature were the highest and the CO2 source was the lowest at the concentration of 38.4 mM. At the concentration of 76.8 mM, the stomatal resistance was the highest and the photosynthetic rate was the lowest. Finally, the highest partial pressure of the water source was related to the concentration of 153.6 mM. Generally, different concentrations of herbicides exhibit different degrees of pheno-physiological traits, and the desired traits can be improved based on these concentrations.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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