Journal of Agricultural Production (2025), *6*(2), 117-125 https://doi.org/10.56430/japro.1678921

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

PRENSIP

https://prensip.gen.tr

RESEARCH ARTICLE

Pre-selection of Imidazolinone-Resistant Canola Plants by Germination and Subsequent Seedling Growth Parameters

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ARTICLE INFO

Article History
Received: 19.04.2025
Accepted: 17.06.2025
First Published: 29.06.2025

Keywords
Brassica napus L.
Germination
Herbicide
Imazamox
Resistant
Root length

ABSTRACT

This study aimed to investigate the potential use of the germination test as a model for screening canola plants for herbicide resistance. Imidazoline-resistant (IMI-R) and susceptible (IMI-S) canola hybrids were germinated in various concentrations of imazamox (0, 0.25, 0.50, 1.00, and 2.00 mM) and clopyralid (0, 0.21, 0.43, 0.85, and 1.72 mM). Germination percentage, mean germination time, germination index, shoot length, root length, seedling fresh and dry weight, and dry matter were analyzed. The results indicated that varying concentrations of imazamox and clopyralid had only a limited effect on the germination parameters in canola hybrids. However, seedling growth parameters such as shoot length, root length, and seedling fresh weight were significantly reduced with increasing concentrations of imazamox and clopyralid. Notably, the differences between IMI-R and IMI-S canola hybrids were evident for these parameters. IMI-S canola hybrid exhibited sensitivity to imazamox, while the IMI-R hybrid showed no significant reduction in growth up to 0.50 mM imazamox. Conversely, the shoot length of the IMI-S hybrid was less affected and was longer than that of the IMI-R hybrid under clopyralid treatment. Of the parameters investigated, root length was the most sensitive to imazamox, which can be used to select herbicide resistance. It was concluded that the suitable imazamox concentrations for selecting imazamox-resistant canola plants during the early growth stage were between 0.50 mM and 1.00 mM. Germination parameters were found to be unsuitable criteria for imazamox resistance, while root length, shoot length, and seedling fresh weight should be considered for selection.

Please cite this paper as follows:

Yaman, E., & Kaya, M. D. (2025). Pre-selection of imidazolinone-resistant canola plants by germination and subsequent seedling growth parameters. *Journal of Agricultural Production*, *6*(2), 117-125. https://doi.org/10.56430/japro.1678921

1. Introduction

Vegetable oils are essential nutrients for maintaining a healthy and balanced human diet. The production of edible oils is necessary to meet the demands of both private households and industries (Safdar et al., 2023). Canola (*Brassica napus* L.) is a globally important oilseed crop used for human consumption, a source of protein-rich animal feed, and a renewable resource for biodiesel production (Zhao et al., 2020). Its seeds and meals contain 40-46% high-quality edible oil and 38-40% protein, respectively (Yaman et al., 2024). The sowing area of canola in Türkiye is expanding because of the benefits of winter sowing, high seed yield, and high oil quality.

Canola production is affected by several adverse environmental conditions, such as drought, extreme

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temperatures, salinity, and weeds. It does not cope with the weeds due to its slow growth habits during emergence and early growth stages (Simic et al., 2011). To mitigate the weed hazard, integrated weed management involves a combination of preventative, mechanical, and chemical methods to maximize seed yield and quality of canola (Grozi, 2023). In the conventional production of oilseed canola, registered selective herbicides do not provide satisfactory control of weeds belonging to the *Brassicaceae* family, such as wild mustard, an ancestor of oilseed rapeseed. To solve this problem, herbicide-resistant canola varieties have been developed using Clearfield® technology. This technology utilizes imidazolinone herbicides and imidazolinone-resistant (IMI-R) canola hybrids (Delchev, 2021a; Grozi, 2023; Pfenning et al., 2012) to eradicate both broadleaf and narrowleaf weeds.

Imidazolinone herbicides are known for their high effectiveness at low doses and broad spectrum of weed control, but they also exhibit high persistence in soils (Balabanova, 2022; Breccia et al., 2020). In traditional oilseed canola cultivation practices, the selective herbicides currently in use have not effectively controlled weeds like *Sinapis arvensis* L., *Raphanus raphanistrum* L., *Capsella bursa-pastoris* L., *Descurainia sophia* (L.) and *Thlaspi arvense* L. (Delchev, 2021b). Therefore, hybrid canola varieties resistant to imazamox have been recently developed and registered to control these weeds (Fernández-Martínez et al., 2009; Malidza et al., 2003). Imazamox is a herbicide belonging to the imidazolinone group and inhibits the activity of the enzyme acetohydroxy acid synthase (AHAS), also known as acetolactate synthase (ALS). It is used post-emergence in leguminous crops, IMI-resistant wheat, sunflower, rice, and canola cultivars (Kaya & Kolsarıcı, 2005).

The selection of canola plants resistant to imazamox is the primary breeding objective for effective weed control. Nevertheless, this process is expensive, time-consuming, and labor-intensive because of the difficulties in identifying the resistant parents. Therefore, this study aimed to use germination and early seedling growth parameters in a laboratory setting to develop a simple, rapid, and efficient pre-selection model for imazamox resistance in canola.

2. Materials and Methods

The experiment was conducted at the Seed Science and Technology Laboratory, Department of Field Crops, Eskişehir Osmangazi University in 2024. Two commercial canola hybrids, the imidazolinone-resistant (IMI-R) hybrid KWS Cyrill CL and the imidazolinone-susceptible (IMI-S) hybrid Riccardo KWS, were used in this study.

The seeds of the canola hybrids were germinated at four concentrations of imazamox: 0.25, 0.50, 1.00, and 2.00 mM. These levels were prepared from Intervix® Pro, which contains 40 g of imazamox per liter. Additionally, the seeds were subjected to four clopyralid concentrations of 0.21, 0.43, 0.85, and 1.72 mM, prepared using Phaeton® containing 100 g of clopyralid per liter. This herbicide is registered for standard canola varieties and is used for comparative effects with imazamox. Distilled water was used as the control treatment.

A standard germination test was conducted to identify imazamox-resistant canola plants by comparing the germination and early seedling growth performance of resistant and susceptible canola hybrids. Four sets of fifty seeds from each canola hybrid were placed between three sheets of filter paper with a 20 × 20 cm dimension. A total of 21 mL of the respective solutions was applied to each replicate. Afterward, the filter papers were rolled and inserted into a zipped plastic bag to avoid moisture evaporation. They were incubated at 20°C for 7 days in the dark. Germination was determined by the appearance of a radicle hook (approx. 2 mm), following ISTA (2018) guidelines. On the last day (7^{th} day), five seedlings from each herbicide level were randomly selected to determine root length (RL), shoot length (SL), and seedling fresh weight (SFW). The seedling dry weight (SDW) was determined after drying at 80°C for 24 h.

Germination percentage (GP), mean germination time (MGT) (ISTA, 2018), germination index (GI) (Salehzade et al., 2009), and dry matter (DM) were calculated using the following equations:

$$GP\ (\%) = \frac{Germinated\ seeds\ at\ final\ day}{Total\ seeds} \times 100 \quad (1)$$

$$MGT\ (day) = \frac{\sum Dn}{\sum n} \quad (2)$$

where D is the number of days since the start of the germination test and n is the number of seeds that germinated on day D.

$$GI = \frac{NGS}{DFC1} + \cdots + \frac{NGS}{DFCx} \quad (3)$$

where NGS is the number of germinated seeds, DFC1 is the days of the first count and DFCx is the days of the final count.

$$DM\ (\%) = \frac{SDW}{SFW} \times 100 \quad (4)$$

2.1. Statistical Analysis

The experiment followed a two-factor factorial arrangement within a completely randomized design (CRD), with four replicates. Data were analyzed using the MSTAT-C software(v. 2.10, Michigan State University), and the differences were compared using Duncan's multiple range test at $p < 0.05$ level.

3. Results

A significant interaction between canola hybrids and imazamox concentrations was detected for all measured parameters except germination percentage (Table 1).

Table 1. Analysis of variance and main effects of imazamox concentrations on the investigated characteristics of IMI-S and IMI-R canola hybrids.

Factors	GP (%)	MGT (day)	GI	SL (cm)	RL (cm)	SFW (mg plant^{-1})	SDW (mg plant^{-1})	DM (%)
Hybrids (A)								
IMI-R	93.1^{b}	2.51^{a}	20.3^{b}	5.09^{a}	6.48^{a}	288^{a}	24.1^{a}	8.51^{b}†
IMI-S	97.8^{a}	2.35^{b}	21.9^{a}	3.60^{b}	2.21^{b}	237^{b}	20.6^{b}	8.82^{a}
Imazamox doses (B)								
0 (distilled water)	94.7	2.81^{a}	18.1^{b}	5.25^{a}	7.43^{a}	289^{a}	22.3^{bc}	7.78^{d}
0.25 mM	97.0	2.41^{b}	21.8^{a}	5.01^{b}	5.00^{b}	288^{a}	22.0^{bc}	7.66^{d}
0.50 mM	95.0	2.42^{b}	21.7^{a}	4.43^{c}	4.44^{c}	259^{b}	21.5^{c}	8.27^{c}
1.00 mM	95.5	2.31^{c}	21.9^{a}	3.89^{d}	3.05^{d}	258^{b}	22.5^{b}	8.71^{b}
2.00 mM	95.0	2.19^{d}	22.3^{a}	3.15^{e}	1.80^{e}	216^{c}	23.5^{a}	10.92^{a}
Analysis of variance								
A	**	**	**	**	**	**	**	*
B	*ns*	**	**	**	**	**	**	**
A×B	*ns*	**	**	**	**	**	*	**

*, **: significant at 5% and 1%, *ns*: non-significant, †: Letter(s) connected with the means denote significance levels at $p<0.05$.

Although the germination percentage of the IMI-S hybrid was higher than that of the IMI-R hybrid, it was not significantly influenced by imazamox concentrations. The mean germination time significantly differed between the hybrids and decreased as imazamox concentration increased. Increasing concentrations of imazamox resulted in a reduction in shoot and root length and seedling fresh weight, whereas the germination index and dry matter content increased.

The germination index was higher in the IMI-S hybrid at all levels of imazamox (Figure 1A). There was a significant difference in shoot and root length between IMI-S and IMI-R canola hybrids at all concentrations of imazamox. The shoot and root length were sensitive to imazamox treatments, as differences between canola hybrids were evident. The shoot length of IMI-S was reduced in the presence of imazamox, while it was significantly lower in the IMI-R hybrid at 1.00 mM (Figure 1C). Imazamox also caused a reduction in root length of the canola hybrids. In the presence of imazamox, the root length of the IMI-S hybrid decreased, but a significant reduction in the IMI-R hybrid was observed at 1.00 mM. The minimum root length of both canola hybrids was obtained at the imazamox dose of 2.00 mM (Figure 1D). Due to the reduction in root and shoot length, the seedling fresh weight also decreased. The IMI-R canola hybrid produced a higher seedling fresh weight than IMI-S, but it was adversely affected at levels higher than 0.50 mM (Figure 1E). There was a significant difference between canola hybrids regarding seedling dry weight in the control. However, imazamox increased the dry weight of the IMI-R canola hybrid and exhibited heavier dry weight at higher concentrations of imazamox. At 2.00 mM imazamox, the IMI-S hybrid had the highest dry matter (Figure 2). However, at 0.50 mM, the dry matter of the IMI-S hybrid significantly decreased. The dry matter of the IMI-R hybrid increased considerably at 0.5 mM imazamox. The effects of imazamox concentrations on seedling growth of two canola hybrids are displayed in Figure 3.

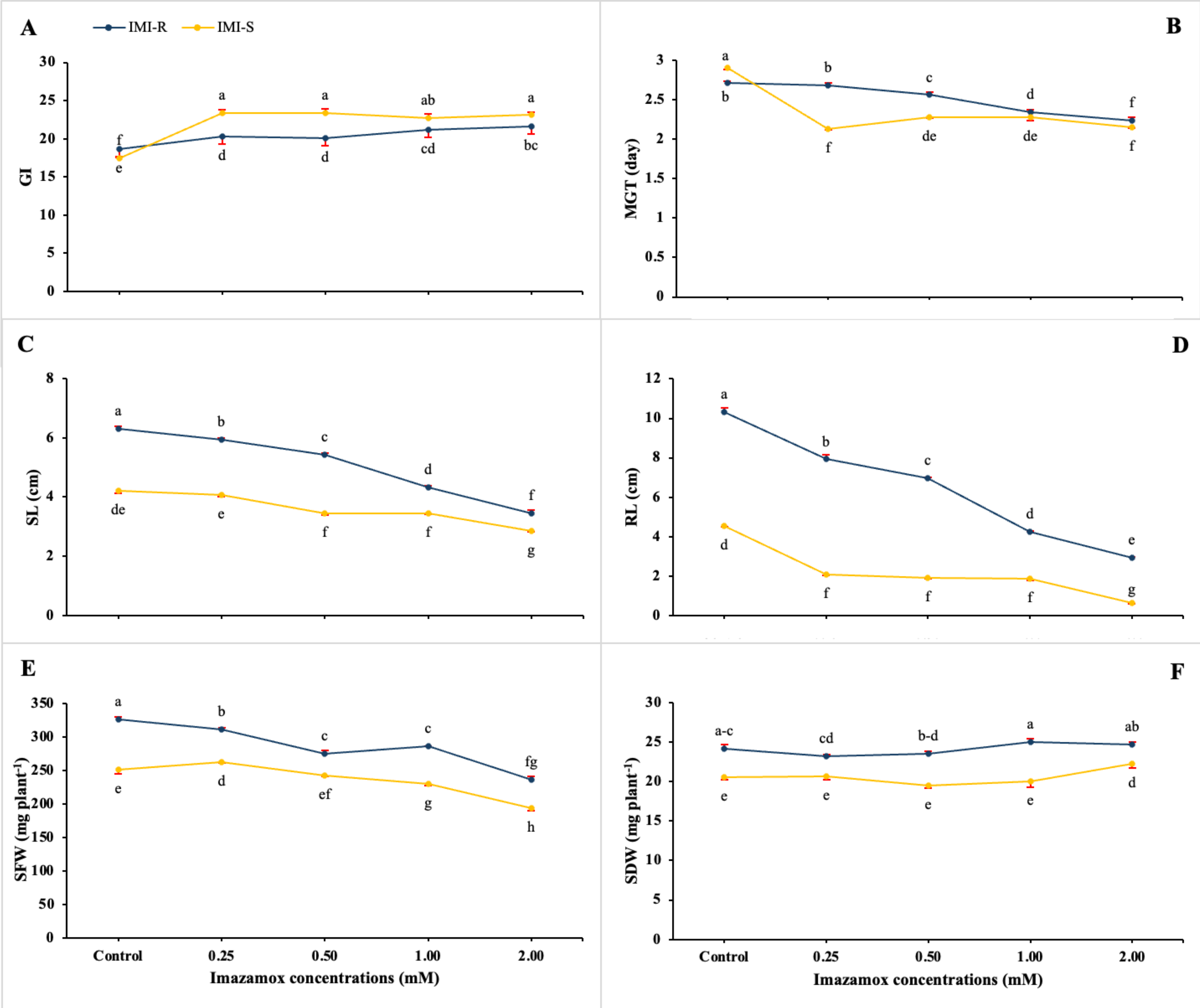

Figure 1. Changes in germination index (GI, A), mean germination time (MGT, B), shoot length (SL, C), and root length (RL, D) of IMI-S and IMI-R canola hybrids under different imazamox concentrations.

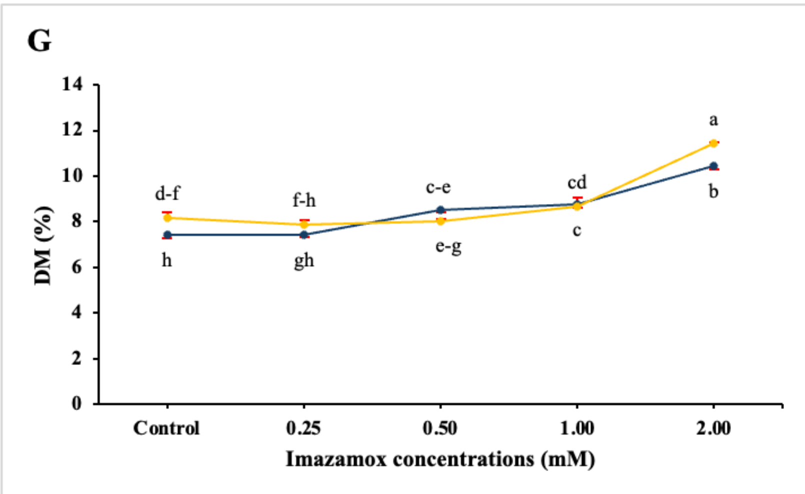

Figure 2. Changes in dry matter (DM, G) of IMI-R and IMI-S canola hybrids under different imazamox concentrations.

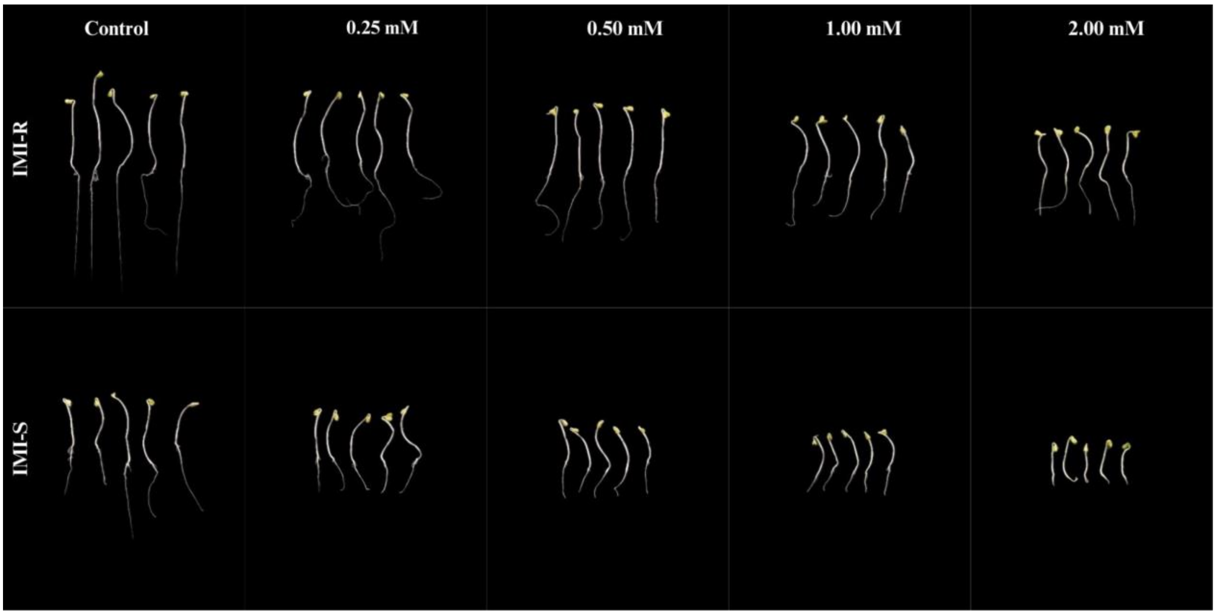

Figure 3. Pictures of seedling growth of IMI-R and IMI-S canola hybrids exposed to various concentrations of imazamox.

Analysis of variance and mean values of canola hybrids and clopyralid concentrations, along with significance levels, were given in Table 2.

Table 2. Analysis of variance and main effects of clopyralid concentrations on the investigated characteristics of IMI-S and IMI-R canola hybrids.

Factors	GP (%)	MGT (day)	GI	SL (cm)	RL (cm)	SFW (mg plant^{-1})	SDW (mg plant^{-1})	DM (%)
Hybrids (A)								
IMI-R	90.2^{b}	2.30	22.6^{a}	4.07^{b}	7.72^{a}	274^{a}	22.9^{a}	8.49^{a}†
IMI-S	96.3^{a}	2.25	22.1^{b}	4.63^{a}	4.35^{b}	246^{b}	17.8^{b}	7.29^{b}
Clopyralid doses (B)								
0 (distilled water)	92.5^{ab}	2.33^{a}	21.5^{bc}	4.90^{ab}	8.33^{a}	286^{a}	20.6	7.14^{c}
0.21 mM	94.7^{a}	2.24^{b}	23.8^{a}	4.99^{a}	6.90^{b}	276^{b}	20.5	7.39^{c}
0.43 mM	93.7^{a}	2.14^{c}	23.5^{a}	4.81^{b}	6.20^{c}	279^{ab}	20.5	7.30^{c}
0.85 mM	95.2^{a}	2.36^{a}	22.1^{b}	3.88^{c}	5.21^{d}	247^{c}	20.3	8.21^{b}
1.70 mM	90.0^{b}	2.31^{ab}	21.1^{c}	3.14^{d}	3.56^{e}	213^{d}	20.0	9.40^{a}
Analysis of variance								
A	**	*ns*	*	**	**	**	**	**
B	**	**	**	**	**	**	*ns*	**
A×B	*	*ns*	**	**	**	**	*ns*	**

*, **: significant at 5% and 1%, *ns*: non-significant, †: Letter(s) connected with the means denote significance levels at $p<0.05$.

Two-way interactions between canola hybrid and clopyralid concentration were significant for all investigated characteristics except for MGT and seedling dry weight (Table 2). The highest germination percentage (95.2%) was achieved at 0.85 mM, while the minimum germination index (21.1) was recorded at 1.72 mM clopyralid. On the other hand, shoot and root length were significantly decreased when the clopyralid levels increased. A similar trend was observed in seedling fresh weight, which also decreased with higher clopyralid concentrations. This finding is surprising for canola, because clopyralid is registered for use with canola varieties. No significant effects of clopyralid levels on seedling dry weight were found. The response of seedling fresh weight of the IMI-R and the IMI-S hybrids to clopyralid was different, with a slight decrease as clopyralid levels increased. In contrast, dry matter increased with higher concentrations of clopyralid. The interaction between canola hybrids and clopyralid concentrations is displayed in Figure 4.

The germination index of canola hybrids varied with increasing concentrations of clopyralid. In the control, the IMI-S hybrid had a lower germination index than the IMI-R hybrid. However, in the presence of clopyralid, the IMI-S hybrid exhibited a higher germination index (Figure 4A). The mean germination time decreased in both canola hybrids as clopyralid levels increased (Figure 4B). Shoot length showed a clear response to clopyralid doses, with the IMI-R hybrid showing longer shoots length under all concentrations of clopyralid (Figure 4C). Increased clopyralid concentrations led to a decline in shoot length. Similarly, both canola hybrids had shorter root length with increasing clopyralid concentrations (Figure 4D and 5). The IMI-R hybrid, however, produced longer roots than the IMI-S hybrid. As shoot and root length decreased, seedling fresh weight decreased with increasing clopyralid levels (Figure 4E), with the IMI-R hybrid showing a heavier weight. The dry matter of canola hybrids was altered by clopyralid concentrations, with a slight increase observed as clopyralid concentrations increased (Figure 4F).

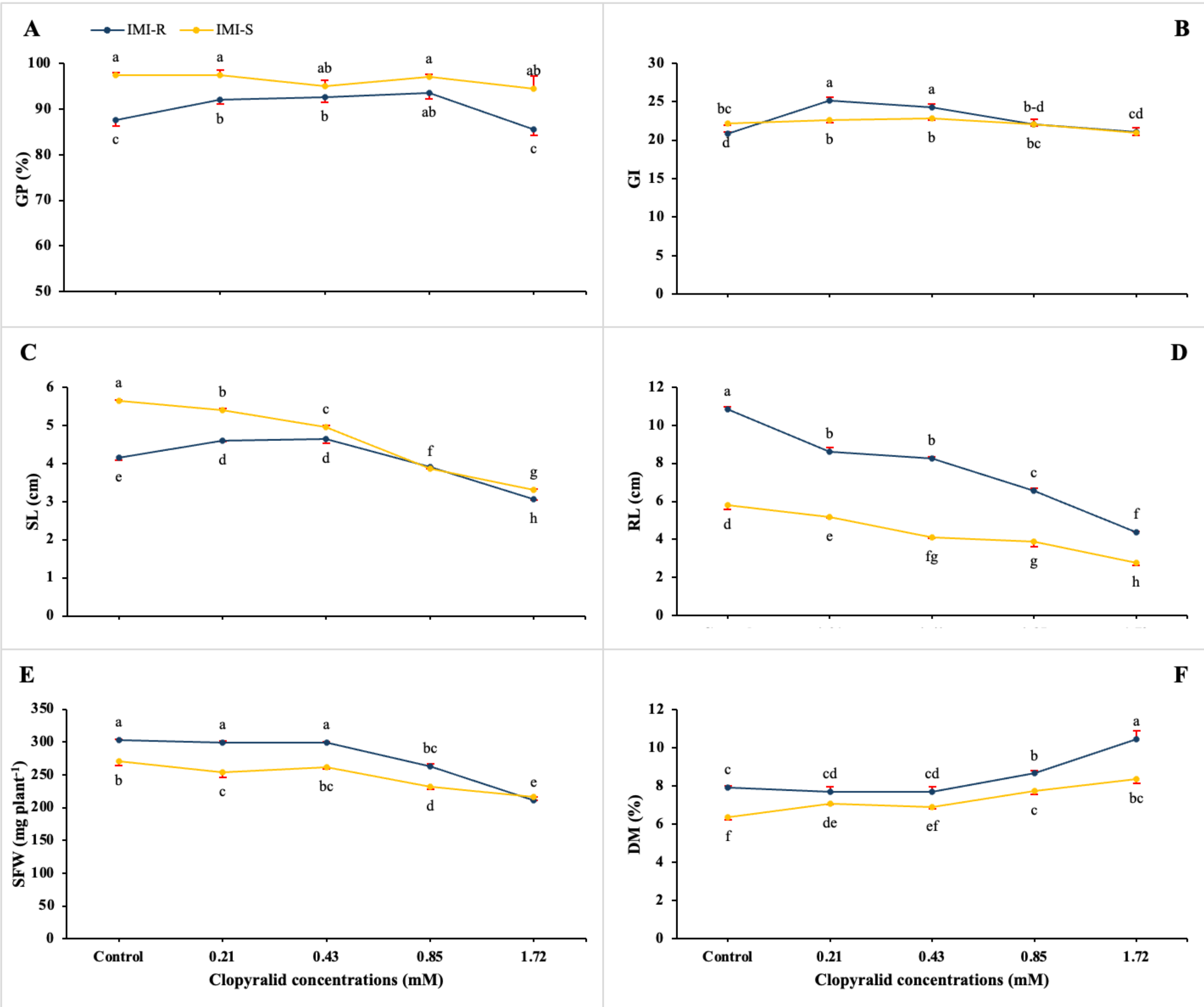

Figure 4. Changes in the investigated parameters of IMI-S and IMI-R canola hybrids under different clopyralid concentrations.

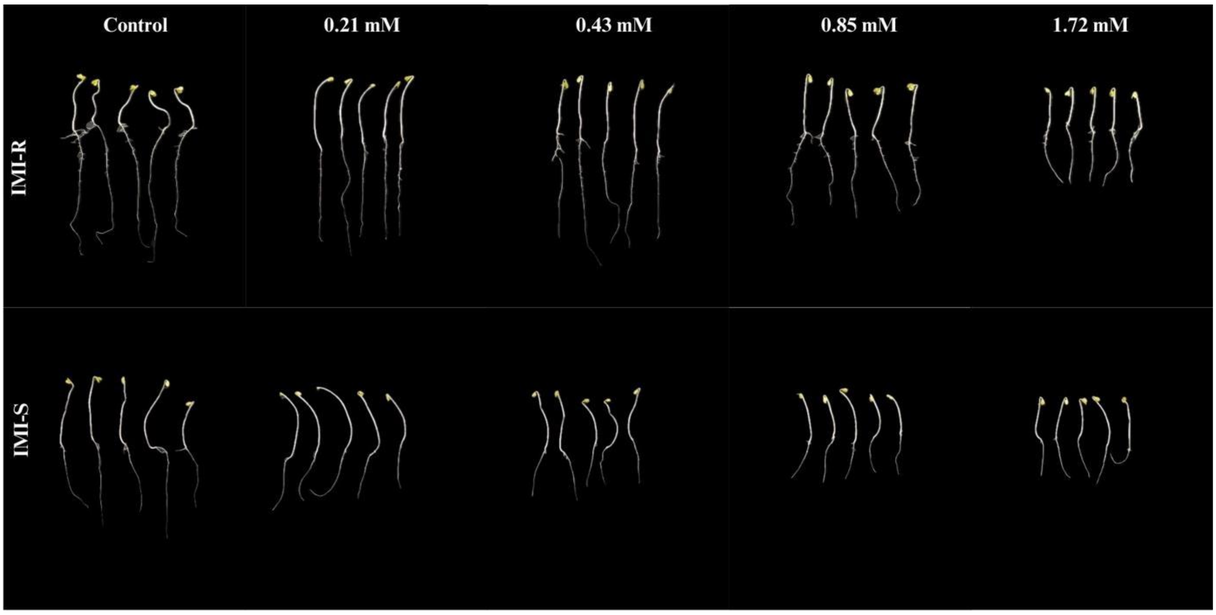

Figure 5. Pictures of seedling growth of IMI-R and IMI-S canola hybrids exposed to various concentrations of clopyralid.

4. Discussion

This study showed that the germination percentage of IMI-S and IMI-R winter canola hybrids was not affected by low, medium, and high doses of imazamox. However, germination was slightly inhibited by a high dose (1.72 mM) of clopyralid. Unlike imazamox, the mean germination time of canola hybrids was undistinguished, while the germination index was reduced as the clopyralid concentrations increased. No significant differences in these parameters were observed between the IMI-S and IMI-R hybrids. Our results are consistent with the findings of Harmancı et al. (2024) in sunflower, who found that the germination percentage of IMI-R and IMI-S hybrids was not influenced by imazamox doses. In the present study, this result argues that the toxicity of imazamox and clopyralid begins after the germinating seeds have absorbed water through the roots.

The seedling growth characteristics, including shoot length, root length, and seedling fresh weight, were severely inhibited by imazamox treatments in the IMI-S canola hybrid. In contrast, no signs of inhibition were observed in the IMI-R hybrid at concentrations up to 1.00 mM imazamox. The sensitivity of root growth in IMI-S canola was particularly noticeable, with inhibition observed at 2.00 mM imazamox. A clear distinction was evident in the root length between the IMI-S and IMI-R hybrids (Figures 1C and 2D, Figure 2E). Similarly, shoot length sensitively responded to imazamox and effectively distinguished the IMI-R hybrid from the IMI-S hybrid. The presence of imazamox led to a decrease in shoot length in the IMI-S hybrid, while it was reduced by the imazamox application of 0.50 mM in the IMI-R hybrid. The seedling fresh weight was also reduced by imazamox, but the response of canola hybrids varied depending mainly on shoot growth. Similar findings were reported in wheat by Breccia et al. (2018) and Haliloğlu et al. (2022), who observed inhibition of root and shoot growth with increasing doses of imazamox.

In the presence of clopyralid, no significant difference between the control and 1.72 mM clopyralid was observed for germination percentage and germination index (Figure 4A, B). Surprisingly, increased clopyralid concentrations restricted shoot length, root length, and seedling fresh weight in both canola hybrids. Furthermore, the sensitivity of shoot growth in the IMI-R hybrid was more pronounced, with depletion observed at all doses of clopyralid. A clear difference was determined between the shoot and root length of the IMI-S and IMI-R hybrids (Figure 4E, F). Compared to imazamox, shoot length did not respond to clopyralid and did not successfully separate the IMI-R from the IMI-S hybrid. The availability of clopyralid resulted in a decrease in shoot length in the IMI-S hybrid, while it first increased and then decreased in the IMI-R hybrid. No dead seedlings were observed in this study. Therefore, the experiment was terminated at 7 days because the canola seedlings in the control group started to deteriorate, which was not enough time for the seedlings to die. When the clopyralid concentrations increased, both IMI-R and IMI-S hybrids showed a dramatic reduction in root length (Figure 4F). This result indicates that clopyralid should not be used as a pre-sowing or pre-emergence herbicide during sowing time because of its inhibitory effects on early seedling growth of IMI-R and IMI-S canola varieties.

5. Conclusion

These results indicate that germination parameters cannot be used as selection criteria in canola plants for herbicide resistance. Apart from imazamox herbicide, clopyralid also caused a decrease in the growth of seedlings in both canola hybrids. The seedling growth was much more sensitive to imazamox, with the IMI-S hybrid being more affected by imazamox than the IMI-R hybrid. Root length, shoot length, and seedling fresh weight are reliable indicators for identifying imazamox-resistant parents in canola germplasm. The optimal concentration of imazamox during the early growth stage of canola was found to be 0.50-1.00 mM. Clopyralid also significantly inhibited seedling growth in both canola hybrids. This inhibition may be attributed to the sensitivity of canola to clopyralid during the early seedling growth stage, making its application not recommended at this stage. Root length and seedling fresh weight may serve as useful indicators for assessing clopyralid resistance in canola.

Acknowledgment

This study is a part of the M.Sc. thesis conducted by the first author, Elif YAMAN, from the Department of Field Crops at the Graduate School of Natural and Applied Sciences, Eskişehir Osmangazi University, Türkiye.

Compliance with Ethical Standards

Ethics committee approval was not required for this study because there was no study on animals or humans.

Conflict of Interest

The authors declare no conflict of interest.

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